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(54) **Title:** AMINO–ALDEHYDE SOLID SUPPORT, LINKER THEREFOR AND METHODS OF PREPARATION AND USE THEREOF

(57) **Abstract**

This invention provides a novel method for solid–phase peptide synthesis wherein a cyclic or linear amino aldehyde is derivatized through linkage to a solid–support, and employed in the production of biologically significant peptides, peptide derivatives, peptidomimetic compounds and analogs thereof. The method of linkage and peptide synthesis, including for combinatorial chemistry, is disclosed.

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TITLE OF THE INVENTION

AMINO-ALDEHYDE SOLID SUPPORT, LINKER THEREFOR AND METHODS OF PREPARATION AND USE THEREOF

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION:

This invention provides a novel method for solid-phase peptide synthesis wherein a cyclic or linear amino aldehyde is derivatized through linkage to a solid-support, and employed in the production of biologically significant peptides, peptidyl aldehydes and derivatives and analogs thereof.

2. BACKGROUND:

In the field of protease inhibition, a number of peptide and peptide analog inhibitors have been developed, as have methods for their synthesis (see U.S. Patent Nos. 5,283,293; 5,367,072; 5,371,072; 5,492,895; 5,514,777; 5,597,804; 5,637,599; 5,646,165; 5,656,600; 5,656,645; 5,681,844; 5,696,231; 5,703,208; 5,714,580; 5,731,413; 5,739,112; all of which are herein incorporated by reference for their disclosure of known protease inhibitors and methods of synthesis and use thereof). Accordingly, there have been significant developments in the field of protease inhibition and the development of structure-activity profiles for a number of classes of protease inhibitors.

It has been recognized that arginine aldehyde exists in solution in one of four principal equilibrium states (as the open arginine aldehyde, as the arginine aldehyde hydrate, and as two amino cyclol forms, i.e. cyclic aldehyde derivatives; see U.S. Patent No. 5,703,208, column 9, herein incorporated by reference; see also Bajusz, S., et al., J. Med. Chem., 33: 1729, 1990, also herein incorporated by reference). In U.S. Patent Nos. 5,514,777 and 5,731,413, methods of synthesis of peptidyl aldehydes, including peptide argininals, were

disclosed. According to that disclosure, a linker comprising ethyl glycolate was employed for immobilization of an argininal cyclol to a solid support. Thus, according to that disclosure, a linker comprising a methylene (-CH_2-) was disclosed. The present invention provides an improved linker and method of synthesis thereof wherein the linker comprises between about one to fifteen, and preferably about five methylene units, and variants thereof. In addition, the present invention provides improved methodology for achieving and cleavage of this linkage. Furthermore, the present disclosure applies this technology to the linkage to solid supports of linear and cyclic aldehydes for synthesis of peptides, peptide analogs and peptidomimetic compounds.

Of particular significance to the field of protease inhibition is the development of synthetic methods of ever increasing efficiency. Accordingly, there is a continued need in the art for novel resins, methods of derivatization and use thereof for solid-phase production of protease inhibitors. The present invention provides a significant improvement on existing solid-phase synthetic methods and resins.

SUMMARY OF THE INVENTION

This invention provides a novel method for solid-phase peptide synthesis wherein a cyclic or linear amino aldehyde is derivatized through linkage to a solid-support, and employed in the production of biologically significant peptides, peptidyl aldehydes and derivatives and analogs thereof.

In one aspect, the invention is a method (Method I) for making a solid support having the formula (I):

(I) R1-O-X-$(CH_2)_n$-Y-CO-NH-SS

wherein:

(a) R1 is derived from R1', a moiety having a cyclic amino aldehyde or derivative thereof at its carboxy-terminus, wherein the moiety R1 optionally comprises a protecting group at one or more positions thereof;

(b) n is an integer from 1 to about 15, provided that n is at least two if both X and Y are bonds, and n is preferably about 5;

(c) SS is a solid support; and

(d) X and Y are independently a bond or -$[Z]_p$-, wherein: p is an integer between 1 and 5, provided that the combination of X, Y and -$(CH_2)_n$- represents a chain equivalent in length to a linear chain of about two to fifteen, and preferably about five carbon atoms; Z is -CH_2CH_2O-, or -C(A)(B)-, wherein A and B may vary in each occurrence of Z, and are independently selected from the group consisting of hydrogen, alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbons, and wherein in combination, A and B may form a five to seven membered ring;

said method comprising the steps of

(a) (i) contacting R1' with a linker of formula OH-X-$(CH_2)_n$-Y-CO_2-R2, wherein R2 is selected from the group consisting of -NH-SS, if the linker is first reacted with a solid support, or H, lower alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbon atoms, and aralkyl of about six to about fifteen carbon atoms, under acid-catalyzed conditions permitting reaction of a carbonyl or nascent carbonyl of R1' with the hydroxy of the linker to form R1-O-X-$(CH_2)_n$-Y-CO_2-R2;

(ii) recovering R1-O-X-$(CH_2)_n$-Y-CO_2-R2 and, when R2 is not -NH-SS, or -H, subjecting the R2 group to hydrolysis to produce a carboxylic acid of formula R1-O-X-$(CH_2)_n$-Y-CO-OH;

(iii) contacting R1-O-X-$(CH_2)_n$-Y-CO-OH with a NH_2-SS, unless the linker was previously reacted with a solid support, under conditions permitting dehydration of the carboxylic acid and amide bond formation between the carboxylic acid and the NH_2-SS to form R1-O-X-$(CH_2)_n$-Y-CO-NH-SS, and

(iv) recovering the R1-O-X-$(CH_2)_n$-Y-CO-NH-SS solid support thus formed.

In a further aspect of this invention, the invention is a method (Method II) for making a solid support having a formula (II):

$R3[-O-X-(CH_2)_m-Y-CO-NH-SS]_2$ (II);

wherein:

(a) R3 is a moiety derived from R3' having a linear chain amino aldehyde, or a derivative thereof, at its carboxy-terminus, wherein the moiety R3' optionally comprises a protecting group at one or more positions;

(b) m is an integer of between about 1 and 15, provided that m is at least two if both X and Y are bonds, and m is preferably about 5;

(c) SS is a solid support; and

(d) X and Y are independently a bond or $-[Z]_p-$, wherein p is an integer between 1 and 5, provided that the combination of X, Y and $-(CH_2)_m-$ represents a chain equivalent in length to a linear chain of one to fifteen, and preferably about five carbon atoms; Z is $-CH_2CH_2O-$, or -C(A)(B)-, wherein A and B may vary in each occurrence of Z, and are independently selected from the group consisting of hydrogen, alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbons, and wherein in combination, A and B may form a five to seven membered ring;

said method comprising the steps of:

(i) contacting the R3' aldehyde with at least a two molar excess, as compared to R3, of a linker of formula:

$OH-X-(CH_2)_m-Y-CO_2-R2$

wherein R2 is selected from the group consisting of -NH-SS, if the linker has previously been reacted with a solid support, or -H, alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbon atoms, and aralkyl of about six to about fifteen carbon atoms, under acid catalyzed conditions permitting reaction of the carbonyl of R3' with the hydroxyl of the linker, to form:

$R3[-0-X-(CH_2)_m-Y-CO_2-R2]_2$,

(ii) recovering the product from (i) and, when R2 is not -NH-SS or -H, hydrolyzing R2 to provide a carboxylic acid,

(iii) contacting the carboxylic acid of (ii) with NH_2-SS, unless the linker was previously reacted with the solid support, under conditions permitting dehydration

of the carboxylic acid and amide bond formation between the carboxylic acid and the NH_2-SS to form a solid support of formula:

$R3[-O-X-(CH_2)_m-Y-CO-NH-SS]_2$ (II); and

(iv) recovering the solid support thus formed.

Accordingly, it is one object of this invention to provide a novel method for linkage of aldehydes to a solid support.

A further object of this invention is to provide a method for derivatization of a cyclic amino aldehyde.

A further object of this invention is to provide novel resins for solid-phase peptide synthesis.

Further objects and advantages of the invention will be apparent from a review of the complete disclosure and the claims appended hereto.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a flow chart representing the linkage of a cyclic aldehyde to a solid support following the procedure of Examples 2 through 7, and synthesis of a growing peptide chain thereon, following the procedures of Examples 8 through 12.

Figure 2 is a flow chart representing the linkage of a cyclic aldehyde to a solid support following the procedure of Examples 13 through 19, and synthesis of a growing peptide chain thereon, following the procedures of Examples 8 through 12.

Figure 3 is a flow chart representing the linkage of a cyclic aldehyde to a solid support which has already been reacted with a linker according to the method of this invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

1. DEFINITIONS:

As used in the present disclosure, the following terms are intended to have the following definitions, unless specifically defined differently within a given context:

Alloc is allyloxycarbonyl

Alloc-Cl is allyl chloroformate

A.M. resin is amino-methylated polystyrene resin

Boc is tert-butoxycarbonyl

Bom is benzyloxymethyl

BOP is benzotriazole-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate

CbZ is benzyloxycarbonyl or carbobenzyloxy

2-ClZ is 2-chlorobenzyloxycarbonyl

DCM is dichloromethane

DIEA is N, N-diisopropylethylamine

DMF is N, N-dimethylformamide

DMSO is dimethylsulfoxide

EDC is 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt

Fmoc is 9-fluorenylmethyloxycarbonyl

HF is hydrogen fluoride

HOBT or HOBt is 1-hydroxybenzotriazole monohydrate

HPLC is high pressure liquid chromatography; high performance liquid chromatography

LAH is lithium aluminum hydride

MS is mass spectrometry

Mts is mesitylene-2-sulphonyl

NMM is N-methylmorpholine(also referred to as 4-methylmorpholine)

NMR is nuclear magnetic resonance spectroscopy

PG is protecting group

PMC is 2,2,5,7,8-pentamethylchroman-6-sulfonyl

PyBOP is Benzotriazole-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate

$(Ph_3P)_4Pd$: tetrakis-(triphenylphosphine)palladium(0)

r.t. or RT is room temperature

TBTU is 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate

TeOC is $Me_3Si(CH_2)OCO$

TFA is trifluoroacetic acid

THF is tetrahydrofuran

TLC or tlc is thin layer chromatography

TFMSA is trifluoromethylsulfonic acid, also commonly referred to as "triflic acid"

TMSOTf is trimethylsilyltrifluoro methane sulfonate

Tos is p-toluenesulfonly also referred to as Tozyl or Ts

Troc is trichloroethoxycarbonyl (an amine protecting group removable with zinc)

NMR designations:

s is singlet

d is doublet

m is multiplet

br is broad peak

t is triplet

q is quartet

SS is a polymeric solid support that is stable in the presence of acids, bases and/or other reagents; a "solid support" is any form of bead or resin typically used in the art of peptide synthesis to provide a "handle" whereby a growing synthetic peptide chain may be made available for synthetic manipulation without the risk of loss in peptide yield typically experienced when such syntheses are conducted in solution; the terms "solid support" and "resin" are used interchangeably. The term "solid support", "SS" or "support" refer to a solid particulate, insoluble material to which a linker moiety of the present invention is linked and from which a peptide or peptide analog may be synthesized. Supports used in synthesizing peptides and peptide analogs are typically substantially inert and nonreactive

with the reagents used in the synthesis of peptides and peptide analogs, particularly once an initial linkage between an aldehyde has been established (i.e., a P1 position has been established), according to the method of this invention. Preferably, a solid support is a cross-linked resin, such as polystyrene.

NH_2-SS is a SS comprising at least one functional amino group available for formation of an amide (peptide) bond.

A cyclic aldehyde is a cyclic α-amino aldehyde, such as a hemiaminal (for arginine), hemiacetal (for Asp/homoSer) and thiohemiacetal (for homoCys); a molecule having a cyclic configuration either bearing an aldehyde moiety or bearing a nascent aldehyde moiety; a prototypical example of this type of molecule is an arginine aldehyde or a peptidyl arginine aldehyde, wherein it has been recognized that arginine aldehyde exists in solution in one of four principal equilibrium states: as the open arginine aldehyde, as the arginine aldehyde hydrate, and as two amino cyclol forms (i.e., cyclic aldehyde derivatives; see U.S. Patent No. 5,703,208, column 9, herein incorporated by reference; see also Bajusz, S., et al., J. Med. Chem., 33: 1729, 1990, also herein incorporated by reference).

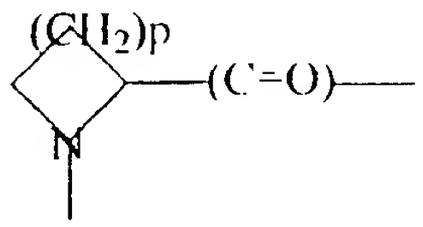
A cyclic aldehyde derivative is any derivative of a cyclic aldehyde; preferably, for purposes of this invention, a cyclic aldehyde derivatized with a blocking group.

A linear aldehyde is any molecule wherein the aldehyde moiety, -CHO, is not cyclized.

The term "amino acid" refers to natural amino acids or unnatural amino acids, and amino acid analogs in their D and L stereoisomers if their structure allows such stereoisomeric forms. Natural amino acids include alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp),

tyrosine (Tyr) and valine (Val). Unnatural amino acids include, but are not limited to azetidinecarboxylic acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, 2,4-diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, n-ethylglycine, N-ethylasparagine, hydroxylysine, all-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, norvaline, norleucine, ornithine and pipecolic acid. Amino acid analogs include, but are not limited to, the natural and unnatural amino acids which are chemically blocked, reversibly or irreversibly, or modified on their N-terminal amino group or their side-chain groups, as for example, methionine sulfoxide, methionine sulfone, S-(carboxymethyl)-cysteine, S-(carboxymethyl)-cysteine sulfoxide and S-(carboxymethyl)-cysteine sulfone, aspartic acid-(beta methyl ester), N-ethylglycine, alanine carboxamide.

The term "amino acid residue" refers to radicals having the structure (1) NH-R-C(O), wherein R typically is -CH(R*)-, wherein R* is H or a carbon containing substituent; or (2)



wherein p is 1, 2, or 3, representing the azetidinecarboxylic acid, proline or pipecolic acid residues, respectively.

A peptide or peptide analog is a molecule comprising at least two amino acids or amino acid analogs linked through peptide (amide) linkages.

A peptidomimetic compound is any compound which structurally resembles or mimics a natural peptidyl array, and compounds comprising such residues; compounds which, although not a natural peptide, in the sense that it either contains no amino acids or

contains amino acid analogs, exhibits a biological activity of a known peptidyl compound.

The terms "good leaving group" or "leaving group" are used herein to define a molecular substituent which, when used in conducting chemical syntheses, exhibits the desirable properties of being labile under defined synthetic conditions, and of being easily separated from synthetic products under defined conditions. Examples of such leaving groups include, but are not limited to, hydrogen, hydroxyl radicals, halogen atoms, p-nitrophenoxide, water, methyl groups, and the like.

The term "protecting group" is used herein to refer to well known moieties which have the desirable property of preventing specific chemical reactions at a site on a molecule undergoing chemical modification intended to be left unaffected by the particular chemical modification, while at the same time being easily removed from the molecule under conditions that do not adversely affect other sites in the modified molecule. Those skilled in the art have a wide variety of known protecting groups to choose from, depending on the nature of the chemical site to be protected. Reference is made, for example, to "Protective Groups in Organic Synthesis", T. Greene, (John Wiley & Sons, Inc., 1991), and to "Solid Phase Peptide Synthesis," Stewart and Young (Pierce Chemical Co., 1984), herein incorporated by reference for this and other purposes. Examples of protecting groups known in the art include, but are not limited to, Cbz, Boc, Alloc, Fmoc, Troc, Teoc ($Me_3Si(CH_2)_2OCO$), PMC, and the like, and others disclosed herein.

The term "alkyl" refers to saturated aliphatic groups including straight-chain, branched-chain and cyclic groups.

The term "alkenyl" refers to unsaturated aliphatic groups having at least one double bond.

The term "aryl" refers to aromatic groups which have at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl

groups, all of which may optionally be substituted with a substituent selected from, but not limited to, lower alkyl of one to ten carbon atoms; alkenyl; nitro; cyano; halo; $-S(O)_q-$, wherein, for purposes of this definition, q is 0, 1, or 2; carboxylic acid or carboxylic acid derivatives, esters amides, and the like.

The term "aralkyl" refers to an alkyl group substituted with an aryl group. Exemplary aralkyl groups include, but are not limited to, benzyl, picolyl, and the like, which may optionally be substituted with a substituent selected from, but not limited to, lower alkyl of one to ten carbon atoms; alkenyl; nitro; cyano; halo; $-S(O)_q-$, wherein, for purposes of this definition, q is 0, 1, or 2; carboxylic acid or carboxylic acid derivatives, esters amides, and the like.

The term "cycloalkyl" refers to an alkyl in which at least a portion of the molecule is in a closed ring configuration. Exemplary cycloalkyl groups include, but are not limited to, cyclohexyl, cyclopropyl, cyclopentyl and cycloheptyl.

The term "heterocycle" refers to any compound having a closed cyclic structure in which at least one atom thereof is other than a carbon atom. For example, cyclic alkyl, cyclic aryl, cyclic aralkyl compounds containing a nitrogen, oxygen or sulfur atom in the cyclic structure are heterocycles.

The term "halo" or "halogen" refers to fluorine, chlorine, bromine and iodine.

The term "non-adverse conditions" describes conditions of reaction or synthesis which do not substantially adversely affect the skeleton of the peptide analog and/or its amino acid (and/or amino acid analog) components. One skilled in the art can readily identify functionalities, coupling procedures, deprotection procedures and cleavage conditions which meet these criteria.

2. DETAILED DISCLOSURE OF THE METHOD OF THIS INVENTION:

In the disclosure which follows, the terms "resin" and "solid support" are used interchangeably, as are the terms "derivatized resin" and "derivatized solid support". In addition, in any given context, where synthesis of a peptide, peptide analog, or peptidomimetic compound is described, where only one of these species is specifically mentioned, synthesis of the other species also intended.

According to this disclosure, we have discovered that provision of a spacer (in figure 1, a pentane spacer is shown) comprising about a five carbon atom linear chain, or a variant thereof having substantially the same spatial effect, substituted or not, along with other enhancements described herein, affords enhanced efficiency in the overall chemical process described herein. It will be appreciated by those skilled in the art, based on this disclosure that the term "about a five carbon atom linear chain" includes linkers wherein two, three, four, five, six or seven carbon atoms, or variants thereof, are linked in a linear chain. Linkers shorter than two or longer than about seven have been found to be less efficient and result in reduced yield of the synthetic process.

I. METHOD OF MAKING SOLID SUPPORT WITH CYCLIC AMINO ALDEHYDE DERIVATIVES:

This invention may best be comprehended with reference to the figures. In figure 1, there is depicted a reaction sequence according to one aspect of this invention, with reference being made to specific exemplary support (see the various example numbers, shown as "Ex." and the relevant example number, at various stages throughout the reaction scheme) provided herein below. According to this scheme, a cyclic aldehyde **100** (exemplified in the reaction scheme by N-α-t-butoxycarbonyl-N^{g}nitro-argininal; those skilled in the art will recognize that solvent, protecting group or reaction condition variations may be made without departing from the procedure defined herein) exhibiting a Boc protected α–amino group is contacted with a preferred linker of this invention

(represented by 6-ethylhydroxyhexanoate) in the presence of an acidic catalyst. After reaction, excess linker is capped by reaction with acetic anhydride and pyridine, or the like, to facilitate purification of the desired product. As a result, an intermediate **110** is provided, with the cyclic aldehyde bonded to the linker. Reduction (e.g., via palladium catalyzed hydrogenation) to the intermediate **120** permits N-omega Alloc protection of the cyclic aldehyde, and hydrolysis of the alkyl group (e.g. ethyl in Figure 1) in subsequent steps, to produce the free-carboxylic acid containing intermediate **130**. Contacting **130** with a resin bearing at least one reactive amino group results in formation of an amide bond between the linker and the resin to form compound **140**. Acid-catalyzed Boc deprotection of the amino group permits standard, solid-phase peptide chemistry to be conducted to produce a peptide, peptide derivative or analog **150**. Deprotection and final cleavage from the resin permits release of the peptide, peptide derivative or analog thereof **160** having a free carboxy-terminal aldehyde functionality. The thus-synthesized compound **160** may then be used directly. Alternatively, the aldehyde functionality may be converted through standard chemical means to a carboxylic acid or other desirable functionality.

By analogy, with reference to figure 2, in a related method according to this invention, an arginine aldehyde is obtained from the parent carboxylic acid **200** by appropriate omega-group protection (e.g., PMC). Alloc or like protection of the α-amino group and conversion to the N-methoxy-N'-methylamide (i.e., Weinreb amide formation from the carboxylic acid moiety) by treatment with O,N-diemthylhdoxylamine or like reagent results in production of compound **210**. Reduction to the aldehyde, reaction with the linker (exemplified by ethyl-6-hydroxyhexanoate in the presence of acetic anhydride or like anhydride and pyridine in a suitable solvent) and purification provides compound **220**. Hydrolysis gives the free acid **230**. Contacting **230** with a resin bearing at least one reactive amino group results in formation of an amide bond between the linker and the resin, followed by Alloc deprotection, to form compound **240**. Standard, solid-phase peptide chemistry is performed with **240** to produce a peptide, peptide derivative or analog **250**. Simultaneous acid catalyzed deprotection and cleavage from the resin

permits release of the peptide, peptide derivative or analog thereof **260** having a free carboxy-terminal aldehyde functionality. The thus-synthesized compound **260** may then be used directly. Alternatively, the aldehyde functionality may be converted through standard chemical means to a carboxylic acid or other desirable functionality.

In yet a further embodiment of the methodology of this invention, with reference to figure 3, there is shown a reaction scheme wherein a starting material **300** representing a trimethylsilyl (TMS) or like protected linker is directly linked with an amino resin and deprotected to form the derivatized resin **310** exhibiting a free, reactive hydroxyl group. Contacting this derivatized resin with nascent cyclic aldehyde **320** (prepared, for example, by treatment of compound **210** from figure 2 with LAH/THF, THF -78°C), results in production of the immobilized cyclic aldehyde **330**. Deprotection of the aldehyde produces the reactive species **340**, with which solid-phase peptide chemistry may be conducted, including but not limited to production of peptide, peptidomimetic protease inhibitors, and preparation of a library of compounds through combinatorial chemical variations in a plurality of different reaction vessels using different monomeric, oligomeric or polymeric condensations with the product **340**.

In view of the foregoing discussion of the figures, it will be appreciated that in one aspect, the invention is a method (Method I) for making a solid support having the formula (I):

(I) R1-O-X-$(CH_2)_n$-Y-CO-NH-SS

wherein:

(a) R1 is derived from R1', a moiety having a cyclic amino aldehyde or derivative thereof at its carboxy-terminus, wherein the moiety R1 optionally comprises a protecting group at one or more positions thereof;

(b) n is an integer from about 1 to about 15, provided that n is at least two if both X and Y are bonds, and n is preferably about 5;

(c) SS is a solid support; and

(d) X and Y are independently a bond or -$[Z]_p$-, wherein: p is an integer between 1 and 5, provided that the combination of X, Y and -$(CH_2)_n$- represents a chain

equivalent in length to a linear chain of about two to fifteen, and preferably about five carbon atoms; Z is $-CH_2CH_2O-$, or -C(A)(B)-, wherein A and B may vary in each occurrence of Z, and are independently selected from the group consisting of hydrogen, alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbons, and wherein in combination, A and B may form a five to seven membered ring;

said method comprising the steps of

(a) (i) contacting R1' with a linker of formula OH-X-$(CH_2)_n$-Y-CO_2-R2, wherein R2 is selected from the group consisting of -NH-SS, if the linker is first reacted with a solid support, or H, lower alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbon atoms, and aralkyl of about six to about fifteen carbon atoms, under acid-catalyzed conditions permitting reaction of a carbonyl or nascent carbonyl of R1' with the hydroxy of the linker to form R1-O-X-$(CH_2)_n$-Y-CO_2-R2;

(ii) recovering R1-O-X-$(CH_2)_n$-Y-CO_2-R2 and, when R2 is not -NH-SS, or -H, subjecting the R2 group to hydrolysis to produce a carboxylic acid of formula

R1-O-X-$(CH_2)_n$-Y-CO-OH;

(iii) contacting R1-O-X-($CH_2)_n$-Y-CO-OH with a NH_2-SS, unless the linker was previously reacted with a solid support, under conditions permitting dehydration of the carboxylic acid and amide bond formation between the carboxylic acid and the NH_2-SS to form R1-O-X-$(CH_2)_n$-Y-CO-NH-SS, and

(iv) recovering the R1-O-X-$(CH_2)_n$-Y-CO-NH-SS solid support thus formed.

It will be appreciated, in view of the foregoing disclosure, that the order of the reaction steps may be varied. Thus, for example, in the reaction schemes shown in figures 1 and 2, the sequence described above is followed. However, in the reaction scheme depicted in figure 3, the linker is first reacted with the solid support to form a derivatized resin, which is then contacted with the aldehyde. These variations naturally come within the scope of this invention, and in the sequence of steps described above, is accommodated by the moiety R2 being a solid support with which the linker has previously been reacted.

The solid support produced according to the method of this invention may be used to advantage in the synthesis of known and novel inhibitors of proteases, including but not limited to serine proteases, aspartyl proteases, and cysteine proteases. Peptides, peptide analogs and peptidomimetic compounds significant to inhibition of enzymes relevant to the blood coagulation pathway may be prepared to advantage according to the methods disclosed herein. Accordingly, novel and known serine protease inhibitors, aspartic acid protease inhibitors, and cysteine protease inhibitors may be prepared using the solid support produced according to the method of this invention. Accordingly, inhibitors of a wide variety of biologically and medically relevant proteolytic enzymes are produced according to the method of this invention. Such protease inhibitors are selected from, but are not limited to: thrombin inhibitors, urokinase inhibitors, inhibitors of factor Xa, elastase inhibitors, inhibitors of Hepatitis C enzymes, chymase inhibitors, trypsin inhibitors, and tryptase inhibitors.

As noted above, R1 is a moiety having a cyclic amino aldehyde, or derivative thereof, at its carboxy-terminus. Suitable R1 moieties include single cyclic amino aldehydes and derivatives thereof, as well as peptide or peptidomimetic side-chains of two or more atoms that have a cyclic amino aldehyde, or derivative thereof, at the carboxy-terminus of the chain.

In a preferred embodiment of the method of this invention, R1 is an arginine aldehyde, an aspartic acid aldehyde, a homoserine aldehyde or a homocysteine aldehyde, and derivatives thereof. Common derivatives of such amino aldehydes are those having the ω-side chain protected with a protecting group (PG) such as Boc, Alloc, PMC, Fmoc, or Teoc. Other protecting groups disclosed herein or known in the art may be used when appropriate.

In a further embodiment of the invention, the alpha and omega heteroatoms of R1 are blocked with PG1 and PG2, respectively, prior to step (i) (see Example 13 through 16 and

Figure 2). In another aspect, the alpha nitrogen of R1 is blocked with PG1 prior to step (i) and the omega nitrogen of R1 is blocked with PG2 after step (ii) (see Example 1 through 5 and Figure 1).

PG1 and PG2 are protecting groups selected to be orthogonal. That is, the selection of PG1 and PG2 is made after considering the intended use of the solid support, such that PG1 or PG2 can be selectively removed. Removal of one PG group avails that deprotected heteroatom for subsequent reaction, while the protected nitrogen remains unavailable for undesired reactions. Examples 8 and 19 describe the removal of the PG from the alpha nitrogen, while the PG on the omega nitrogen is retained to protect that nitrogen during subsequent reactions (see Examples 10 and 20). Preferred combinations of PG1 and PG2 include the following PGs, which can be either PG1 or PG2: Boc in combination with Fmoc, Alloc, or Teoc; Fmoc in combination with Alloc, Teoc, or PMC; Alloc in combination with Teoc or PMC.

A preferred NH_2-SS is a resin having a functional amine group, such as amino methylated polystyrene resin, benzhydrylamine resin, and 4-methylbenzhydrylamine resin. Other resins known in the art having at least one functional amine group may be used according to the methods disclosed herein.

In view of the foregoing detailed disclosure, it will be appreciated that step (i) set forth above proceeds under acid-catalyzed conditions, such as those disclosed in figures 1 and 2, Examples 3 through 5, Example 16, and elsewhere throughout this disclosure and according to methods known in the art. Step (ii) comprises hydrolysis, which is conducted under conditions such as those reflected in Example 6, Example 17, and according to methods known in the art for this type of chemical transformation. Step (iii) comprises dehydration conditions, such as those disclosed in Example 7, Example 18, and conditions known in the art for this type of chemical transformation.

In a preferred embodiment according to this invention, linker OH-$(CH_2)_n$-CO_2-R2 has n = 4 to 10, and preferably n = 5. Preferred R2 groups include but are not limited to lower (about one to ten carbon atoms) alkyl, alkenyl, and aralkyl. An especially preferred R2 group is ethyl. An especially preferred linker is OH-$(CH_2)_5$-CO_2-ethyl.

Where X and Y are not bonds, either or both moieties may be alkyl or substituted alkyl groups, provided that if substituted, reactive moieties likely to interfere in standard peptide synthetic regimens are not preferred. In addition, it is important not to introduce a functionality in X or Y that could undesirably destabilize the ether linkage produced upon linkage of an aldehyde to a solid support by means of the linker of which X and Y form a part. Preferably, in combination, X, Y and the repeating methylene unit combine to form a spacer that is inert and which provides a length similar to that which an alkyl spacer of about five carbon atoms would provide.

II. METHOD OF MAKING SOLID SUPPORT WITH AMINO ALDEHYDES HAVING LINEAR SIDE CHAINS AND DERIVATIVES THEREOF:

In a further aspect of this invention, a method (Method II) for making a solid support having a formula (II):

$$R3[\text{-}O\text{-}X\text{-}(CH_2)_m\text{-}Y\text{-}CO\text{-}NH\text{-}SS]_2 \quad (II);$$

wherein:

(a) R3 is a moiety derived from R3' having a linear chain amino aldehyde, or a derivative thereof, at its carboxy-terminus, wherein the moiety R3' optionally comprises a protecting group at one or more positions;

(b) m is an integer of between about 1 and 15, provided that m is at least two if both X and Y are bonds;

(c) SS is a solid support; and

(d) X and Y are independently a bond or -$[Z]_p$-, wherein p is an integer between 1 and 5, provided that the combination of X, Y and -$(CH_2)_m$- represents a chain equivalent in length to a linear chain of one to about fifteen, and preferably about

five carbon atoms; Z is $-CH_2CH_2O-$, or -C(A)(B)-, wherein A and B may vary in each occurrence of Z, and are independently selected from the group consisting of hydrogen, alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbons, and wherein in combination, A and B may form a five to seven membered ring;

said method comprising the steps of:

(i) contacting the R3' aldehyde with at least a two molar excess, as compared to R3, of a linker of formula:

$$OH\text{-}X\text{-}(CH_2)_m\text{-}Y\text{-}CO_2\text{-}R2$$

wherein R2 is selected from the group consisting of -NH-SS, if the linker has previously been reacted with a solid support, or -H, alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbon atoms, and aralkyl of about six to about fifteen carbon atoms, under acid catalyzed conditions permitting reaction of the carbonyl of R3' with the hydroxyl of the linker, to form:

$$R3[\text{-}0\text{-}X\text{-}(CH_2)_m\text{-}Y\text{-}CO_2\text{-}R2]_2,$$

(ii) recovering the product from (i) and, when R2 is not -NH-SS or -H, hydrolyzing R2 to provide a carboxylic acid,

(iii) contacting the carboxylic acid of (ii) with NH_2-SS, unless the linker was previously reacted with the solid support, under conditions permitting dehydration of the carboxylic acid and amide bond formation between the carboxylic acid and the NH_2-SS to form a solid support of formula:

$$R3[\text{-}O\text{-}X\text{-}(CH_2)_m\text{-}Y\text{-}CO\text{-}NH\text{-}SS]_2 \text{ (II); and}$$

(iv) recovering the solid support thus formed.

In a preferred embodiment according to this invention, linker $OH\text{-}(CH_2)_m\text{-}CO_2\text{-}R2$ has m = 4 to 10, and preferably m = 5. Preferred R2 groups include but are not limited to lower (about one to ten carbon atoms) alkyl, alkenyl, and aralkyl. An especially preferred R2 group is ethyl. An especially preferred linker is $OH\text{-}(CH_2)_5\text{-}CO_2$-ethyl.

Where X and Y are not bonds, either or both moieties may be alkyl or substituted alkyl groups, provided that if substituted, reactive moieties likely to interfere in standard peptide synthetic regimens are not preferred. In addition, it is important not to introduce a functionality in X or Y that could undesirably destabilize the ether linkage produced upon linkage of an aldehyde to a solid support by means of the linker of which X and Y form a part. Preferably, in combination, X, Y and the repeating methylene unit combine to form a spacer that is inert and which provides a length similar to that which an alkyl spacer of about five carbon atoms would provide.

The solid support prepared according to the method of this invention may be used to advantage in the synthesis of known and novel inhibitors of proteases, including, but not limited to, proteases significant to the blood coagulation pathway. Accordingly, novel and known thrombin inhibitors, inhibitors of factor Xa, urokinase inhibitors, Hepatitis C enzymes, chymase, prostate specific antigen (PSA), Factor VIIa, elastase, trypsin, and the like may be prepared using the solid support produced according to the method of this invention.

III. PROTECTING GROUPS:

It will be appreciated, particularly in the synthesis of peptides, peptide analogs and peptidomimetic compounds, that where reactive moieties are present, as in reactive amino acid side chains, if reactions at that reactive moiety is not desired, methods are known for protecting such sites. Accordingly, where a reactive heteroatom exists in an amino acid side chain, or where reaction at an amino acid or amino acid analog's amino-terminal nitrogen is to be avoided, the following guidelines and procedures will apply. However, where side chains are non-reactive, those skilled in the art will appreciate that protection is not required (e.g. methylcysteine is already effectively protected; the phenylalanine side chain is relatively non-reactive). In particular, with regard to an argininal moiety, wherein the side chain has a guanidino group (the arginine side chain is:

$-(CH_2)_3-NH-(C=NH_2^+)-NH_2$, wherein the moiety $-NH-(C=NH_2^+)-NH_2$ represents the guanidino functionality), single or double protection of the side chain may be desirable or essential in order to achieve a desired reaction at another side, e.g., at the amino-terminal nitrogen, without production of a plurality of unwanted side-chain reactions. It will also be appreciated that while the argininal moiety exists in solution in various tautomeric forms, appropriate side-chain protection may be employed to keep the side chain in a linear form.

In practicing the methods of the present invention, the following considerations apply to the selection of α-amino protecting groups, omega side chain protecting groups, and carboxy protecting groups. In selecting suitable α-amino protecting groups (PG1) to be used during the synthesis of compounds of this invention α-amino protecting group should:

(i) render the α-amino function inert under the conditions employed in the coupling reaction;

(ii) be readily removable after the coupling reaction under conditions that will not remove side chain or carboxy terminus protecting groups; and

(iii) eliminate the possibility of racemization upon activation prior to coupling.

A suitable α-amino protecting group may be selected from the group consisting of acid labile α-amino protecting groups (cleavage conditions are noted, as well, in brackets or text):

(a) Triphenylmethyl (trityl) group is cleaved under very mild acid conditions [1% TFA].

(b) tert-Butyloxy carbonyl (Boc), t-amyloxycarbonyl, adamantyloxycarbonyl, 4-methoxy benzyloxycarbonyl; these protecting groups require moderately strong acids such as trifluoroacetic acid, hydrochloric, or boron trifluoride in acetic acid for their removal.

(c) Benzyloxycarbonyl (CBz), 2-chlorobenzyloxycarbonyl (2-ClZ), cycloalkyloxycarbonyl, and isopropyloxycarbonyl, require stronger acids, such as hydrogen fluoride, hydrogen bromide or boron trifluoroacetate in trifluoro acetic acid for their removal. The CBz and the 2-ClZ groups may most conveniently be cleaved

by hydrogenation over palladium on carbon in methanol. A suitable α-amino protecting group also may be selected from the group consisting of base labile α-amino protecting groups. For instance, Fluorenylmethyloxycarbonyl (Fmoc) may be cleaved by using 20% piperidine/DMF or excess diethylamine in THF.

Allyloxycarbonyl (Alloc) may be cleaved by Pd (0) catalyst transfer of the allyl group to an acceptor nucleophile such as morpholine, dimedone, tributyl tin hydride and N-methyl aniline. Preferred α-amino protecting groups (PG) include Boc, Fmoc, Alloc, and Cbz.

An amino acid side-chain protecting group should:

(i) render the protected side chain functional group inert under the conditions employed in the coupling reaction;

(ii) be stable under the conditions employed in removing the α-amino or the carboxy terminus protecting groups, and

(iii) be readily removable upon completion of the desired peptide under reaction conditions that will not alter the structure of the peptide chain. A suitable amino acid side chain protecting group may be selected from the group consisting of (methods for cleavage of these protecting groups are shown in brackets []):

(a) for protection of lysine amino groups, any of the groups mentioned above for the protection of α-amino protecting groups are suitable.

(b) for protection of arginine guanidino group, the preferred protecting groups include nitro [H2/Pd/C, HF], benzyloxycarbonyl (CBz) [HF, TFMSA, TMSOTf, H2/Pd/C], tert-butyloxycarbonyl (Boc) [TFA], 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) [TFA], 2,3,6-trimethyl-4-methoxyphenylsulfonyl (Mtr) [TFA], p-toluenesulfonyl (Tos) [HF, TFMSA], mesitylene-2-sulphonyl (Mts) [HF, TFMSA], allyloxycarbonyl (Alloc) [Pd(0), morpholine or dimedone].

(c) For protection of serine and threonine hydroxyl groups, protecting groups include trityl [1% TFA], tert-butyl [TFA], benzyl, and substituted benzyl groups such as 4-methoxybenzyl, 4-chlorobenzyl, 2-chlorobenzyl, and 2,6-dichlorobenzyl which are cleaved by a similar method [HF, TFMSA,H2/Pd/C].

(d) For protection of tyrosine phenolic group, protecting groups such as tert-butyl [TFA], trityl [1% TFA], benzyl, 2-bromobenzyl and 2,6-dichlorobenzyl, all cleaved by the same reagents [HF, TFMSA, H2/Pd/C], are suitably employed.

(e) For protection of aspartic and glutamic acid side chain carboxy group, protecting groups include methyl [OH-, H+], ethyl [OH-, H+], t-butyl [TFA], allyl [Pd(0), morpholine], cyclohexyl [HF, TMSOTf], or benzyl groups [HF, TFMSA, TMSOTf, H2/Pd/C].

(f) For protection of asparagine and glutamine side chain, protecting groups include trityl [TFA] and xanthyl [TFA].

(g) For protection of histidine imidazole group, suitable protecting groups include 2,4-dinitrophenyl (Dnp) [thiophenol], trityl [TFA], benzyloxymethyl (Bom) [HF, TFMSA, TMSOTf, H2/Pd/C], p-toluene sulfonyl (Tos) [HF, TFMSA], and benzyloxycarbonyl (Cbz) [HF, H2/Pd/C].

(h) For protection of cysteine sulfhydryl group, suitable protecting groups include trityl [TFA], 4-methylbenzyl (pMeBzl) [HF, TFMSA], 4-methoxybenzyl (pMeOBzl) [HF, TFMSA], acetamidomethyl (Acm) [I2, Hg2+], tert-Butyl (tBu) [Hg2+].

(i) For protection of tryptophan indole group, suitable protecting groups include formyl [10% piperidine in DMF, followed by HF] and tert-butyloxycarbonyl (Boc) [TFA].

A carboxy terminus protecting group should:

(i) render the protected functional group inert under the conditions employed in the coupling reaction,

(ii) be stable under the conditions employed in removing the α-amino or the side chain protecting groups, and

(iii) be readily removable upon completion of the desired peptide under reaction conditions that will not alter the structure of the peptide chain. For the protection of the carboxy terminus of amino acids suitable protecting groups include the esters methyl [OH-, H+], ethyl [OH-, H+], tert-butyl [TFA], benzyl [OH-, H2/Pd/C] and allyl [Pd(0), morpholine] groups.

3. METHOD OF USING THE SOLID SUPPORTS:

(A) Solid-Phase Peptide Synthesis:

Solid-phase synthesis is useful for the production of relatively small amounts of certain compounds. Solid-phase synthesis also is useful for the synthesis of a library of compounds, each differing from the other in one or more variables. As with the conventional solid-phase synthesis of peptides, reactors for the solid-phase synthesis of peptidyl aldehydes, including peptide argininals, are comprised of a reactor vessel with at least one surface permeable to solvent and dissolved reagents, but not permeable to synthesis resin of the selected mesh size. Such reactors include glass solid phase reaction vessels with a sintered glass frit, poylpropylene tubes or columns with frits, or like reaction vessels known in the art or commercially available. The type of reactor chosen depends on volume of solid-phase resin needed, and different reactor types might be used at different stages of a synthesis.

According to the present invention, the linker and solid supports prepared according to Methods I and II (collectively referred to as "the invented solid supports") may be employed in the solid phase synthesis of peptide or peptidyl aldehyde compositions. Preferred uses include use for the synthesis of peptide or peptidyl aldehyde inhibitors of one or more serine proteases. An especially preferred use of the invented solid supports is use for the synthesis of thrombin inhibitors, Factor Xa inhibitors, and urokinase inhibitors (see Example 12 for preparation of a thrombin inhibitor, Example 21 for preparation of a factor Xa inhibitor, and Example 9 for preparation of a urokinase inhibitor using the solid support of this invention and the methods disclosed herein for preparation thereof).

Because the linker of formula (I) or (II) comprises an ether linkage, it is important to bear the chemical features and reactivities in mind upon use of the method of this invention to conduct solid-phase chemistry. In general, where cleavage of the linkage is to be

avoided, it is important to maintain relatively low acid and minimize contact with nucleophiles, particularly in the presence of acid. Keeping reagents and solvents dry (i.e., low water content) is critical. It will also be appreciated that when the method of the present invention is utilized for production of peptide aldehydes, linkage of pre-formed oligomeric peptide units is preferred, due to the susceptibility of the ether linkage to acid conditions, which are similar, for example, to conditions required in the sequential monomeric addition of amino acids (i.e., the conditions for removal, for example, of Boc protecting groups are similar to the conditions under which the ether linkage may become labile). Accordingly, synthesis of relatively short peptides under mild acid conditions or linkage of pre-formed peptide blocks is preferred.

Thus, a further aspect of this invention is the preparation of a derivatized resin by linking to the group R1 or R3 one or more amino acids, amino acid analogs, peptides or peptide analogs to form a peptide or peptidomimetic chain of amino acids or amino acid derivatives comprising the cyclic aldehyde or nascent aldehyde R1 or R3 at the carboxy terminus thereof. As a result of practicing this aspect of the invention, a product represented by formula (III) or (IV):

(III) $(res)_t$-R1-O-X-$(CH_2)_n$-Y-CO-NH-SS,

(IV) $(res)_t$-R3[-O-X-$(CH_2)_m$-Y-CO-NH-SS]$_2$

is produced wherein the moiety $(res)_t$- represents a peptide or peptide analog comprising "t" residues, wherein "t" is an integer between about one and fifty. Preferably, "t" is an integer between about one and about 10.

Accordingly, a product selected from any of the following is produced according to this aspect of the invented method:

N-α-t-butoxycarbonyl-N^g-nitro-argininal (6-hexanoic acid ethyl ester) cyclol;

N-α-t-butoxycarbonyl-argininal (6-hexanoic acid ethyl ester) cyclol, acetate salt;

N-α-t-butoxycarbonyl-N-omega-allyloxycarbonyl-argininal (6-hexanoic acid ethyl ester) cyclol;

N-α-t-butoxycarbonyl-N-δ-Alloc- N-α-t-butoxycarbonyl-N-omega-allyloxycarbonyl-argininal (6-hexanoic acid) cyclol;

N-α-t-butoxycarbonyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol;

N^{δ}-Alloc-Arginylcyclo-O-6-hydroxylhexanoyl-amino methylated polystyrene;

N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol;

N-α-Fmoc-alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol;

alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol;

N-α-Fmoc-D-seryl(O-t-butyl)-alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol;

D-seryl(O-t-butyl)-alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol;

carbamate analogs of D-seryl(O-t-butyl)-alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol;

isobutyloxycarbonyl-D-seryl(O-t-butyl)-alanyl-N-d-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol;

carbamate analogs of D-seryl(O-t-butyl)-alanyl- argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol;

benzylsulfonamido-7Lactam-Gly-N^{δ}-Alloc-Arginylcyclo-O-6-hydroxylhexanoyl-amino methylated polystyrene;

Benzylsulfonamido-7Lactam-Gly-Arginylcyclo-O-6-hydroxyhexanoyl-amino methylated polystyrene;

N-α-Alloc-N^{δ}-PMC-Arginylcyclo-O-Ethyl-6-hydroxylhexanoate;

N-α-Alloc-N^{δ}-PMC- Arginylcyclo-O-6-hydroxylhexanoic acid;

N-α-Alloc-N^{δ}-PMC- Arginylcyclo-O-6-hydroxylhexanoyl-amino methylated polystyrene resin;

N^d-PMC-Arginylcyclo-O-6-hydroxyhexanoyl-amino methylated polystyrene resin; and Benzylsulfonamido-dArg(NO_2)-Sarcosine-Gly- N^δ-PMC-Arginylcyclo-O-6-hydroxyhexanoyl amino methylated polystyrene resin.

(B) Combinatorial Chemistry:

The invented solid supports and linker also are useful in the field of combinatorial chemistry and development of chemical libraries. With reference to the following disclosure, it will be appreciated that preferred methods within this aspect of the invention result in the development of combinatorial libraries having an aldehyde in the P1 position, especially argininals:

$$P_4\text{-}P_3\text{-}P_2\text{-}P_1$$

Those skilled in the art will recognize that while a molecule bearing four residues is represented, peptides or peptide analogs of any desired length may be prepared according to this method by repeating the coupling steps as many times as necessary. According to this aspect of the invention, a library is designed wherein the P_1 residue is kept constant while residues P_4, P_3, and P_2 are varied and incorporated into, for example, peptides and peptidomimetic P_1 aldehydes. Using the novel resin of this invention, multiple reactions are carried out in parallel. The P_1 site is first incorporated onto the resin bearing the linker as disclosed herein, followed by the variations in the P_4, P_3, and P_2 residues, thereby forming a library of peptide or peptidomimetic compounds available for structure-activity analyses in any of a number of *in vitro* or *in vivo* assay systems, including protease inhibition assays.

As will be appreciated by those skilled in the art, this process of library formation and parallel synthesis may be carried out in a number of known formats. In one embodiment, the synthesis is conducted in Whatman mini columns, or the like, wherein standard

peptide synthetic methods known in the art are used to extend the peptide chain, with each subsequent coupling being achieved at the carboxy terminus of each added residue. Following synthesis, the multiple peptide variants are cleaved from the resin, isolated, and tested for biological activity. Likewise, with the aid of the derivatized resin of this invention, automated synthesis of a library of peptides or peptide analogs may be conducted in any commercially available peptide synthesizer.

Thus, this aspect of the invention represents a method for making a library of peptides or peptide analogs comprising the steps of:

(a) in each of a series of separate containers or reaction vessels, contacting an aldehyde with the free hydroxyl of the linker as disclosed herein under acid conditions;

(b) hydrolyzing the product of step (a) to produce a free carboxylic acid moiety at the terminus of the linker;

(c) contacting the product of step (b) with a resin comprising at least one functional amino group to form an amide bond, thereby immobilizing said aldehyde as a P1 residue linked to said resin through said linker;

(d) in each of said series of separate containers or reaction vessels, contacting different amino acid residues or amino acid analog residues with the thus immobilized aldehyde under conditions permitting formation of a peptide bond so as to produce a series of P_2 residues, same or different, in each of said series of separate containers or reaction vessels; and

(e) repeating step (d) as many times as required to generate a peptide or peptide analog of the desired number of residues, with appropriate intermediate steps of protection and deprotection of reactive groups present on the growing peptide or peptide analog chain.

Standard peptide synthetic methods may be employed in this process, subsequent to the initial formation of the aldehyde-linker-resin linkage. Subsequent to cleavage from the resin, the thus synthesized library of peptides or peptide analogs bearing the original aldehyde as the P_1 residue may then be purified and tested for biological activity.

C. Cleavage Conditions:

Standard methods for cleavage of peptide aldehyde or peptide aldehyde analogs prepared according to the method of this invention are not preferred. Standard cleavage conditions, including for example, treatment with HF; TFA; TFA:H_2O (e.g. 9:1); TFA:DCM:H_2O (e.g. 5:4:1), while utilizable, result in lower than optimal yields due to dehydration of the aldehyde moiety, such as conversion of a cyclic argininal tautomer to a covalently cyclized dehydration adduct. Serine residue dehydration may also occur under standard cleavage conditions, and this, too, is undesirable. Accordingly, we have unexpectedly discovered that optimization of the cleavage conditions to include a TFA:DCM:H_2O mixture of about 6:3:1 for about two hours provides an optimized yield. Variations of these conditions, as in minor variations in the time or TFA, DCM or H_2O ratios , or use of solvents or acids having similar properties, come within the scope of this aspect of the invention.

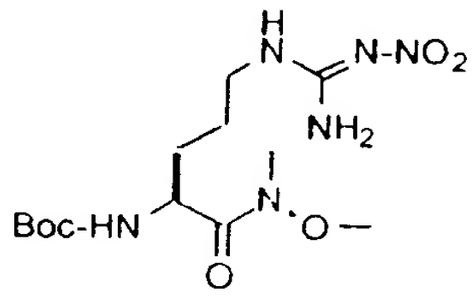
4. SPECIFIC EXEMPLARY SUPPORT:

Having generally described the invention with respect to the preferred embodiments thereof, the following specific exemplary disclosure is provided.

Unless otherwise noted, all materials employed in the following examples are readily available from commercial sources.

Example 1

Preparation of N-α-t-butoxycarbonyl-N^g-nitroargininyl-N-methoxy-N-methylamide



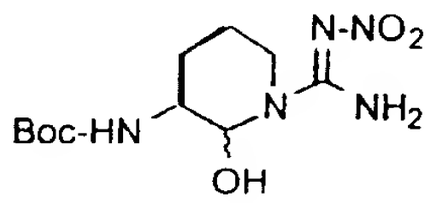
4-methylmorpholine (41.2 g, 407 mmol, 1.3 equiv.) was added to a solution of N-α-t-butoxycarbonyl-N^g-nitroarginine (100 g, 313 mmol), N,O-dimethylhydroxylamine hydrochloride (61.2 g, 626 mmol, 2 equiv.), EDC (77.9 g, 407 mmol, 1.3 equiv.), and 1-hydroxybenzotriazole (55.1 g, 407 mmol, 1.3 equiv.) in anhydrous acetonitrile (523 mL). The reaction was stirred at room temperature over 16 h and solvents were removed in vacuo. The residue was partitioned between ethyl acetate and water. The aqueous phase was re-extracted with ethyl acetate (2 X). The combined organic layers were washed with 1N HCl (1 X), water (1 X), saturated sodium bicarbonate (1 X), water (1 X), and then dried (magnesium sulfate), filtered, and concentrated in vacuo. The title compound was obtained in 78% mass recovery as a white foam and was used without further purification. TLC of the products indicated satisfactory purity. $R_f = 0.21$ (10% methanol/ dichloromethane).

MS ($M + H^+$) = 363.0, calculated (MW) = 362.2

^1H NMR (400 MHz, $CDCl_3$): δ 1.47 (s, 9H), 1.55-1.68 (m, 2H), 1.71-1.83 (br, 2H), 3.23 (s, 3H), 3.25-3.35 (m, 1H), 3.55-3.68 (m, 1H), 3.75-3.80 (s, 3H), 4.66 (t, 1H), 5.63 (d, 1H).

Example 2

Preparation of N-α-t-butoxycarbonyl-N^g-nitro-argininal



To a solution of the compound of Example 1 (25 g, 68.9 mmol, 1 equiv.), in anhydrous tetrahydrofuran (300mL) in a 1 Liter 3 necked round bottomed flask was added dropwise 1M lithium aluminum hydride/tetrahydrofuran (100mL, 1.45 equiv.) at -78°C over 30 min under nitrogen atmosphere. The reaction was kept at -78°C with stirring for 1 h, and then allowed to stir at room temperature for 20-30 min. A thick slurry was observed.

The reaction mixture was once again cooled to -78°C and quenched slowly with 2M potassium bisulfate (100mL). The precipitate was filtered out and washed with tetrahydrofuran (200mL). The combined filtrate was concentrated in vacuo. The crude residue was partitioned with ethyl acetate and water. The organic phases were washed with 0.5 N HCl, saturated sodium bicarbonate, and brine. The residue was dried over magnesium sulfate and filtered. Evaporation of the filtrate gave 15.9 g (76% yield) of product as a white solid. R_f = 0.13 (50% hexane/ethyl acetate). The desired product was judged pure by TLC.

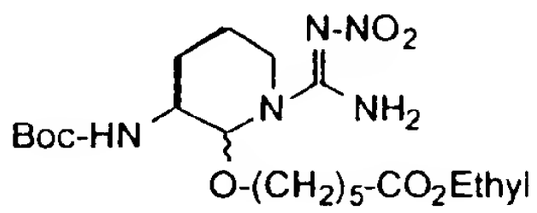
MS: $(M + H^+)$ = 304.0, calculated (MW) = 303.1

^{1}H NMR (400 MHz, $CDCl_3$): d 1.47 (s, 9H), 1.55-1.68 (m, 2H), 1.71-1.83 (m, 2H), 3.19-3.35 (m, 2H), 4.41-4.48 (m, 1H), 5.82 (s, 1H).

Alternatively, the title compound can be made following the procedure set forth in Example 2 of U.S. 5,731,413.

Example 3

Preparation of N-α-t-butoxycarbonyl-N^g-nitro-argininal (6-hexanoic acid ethyl ester) cyclol



To a stirred solution of the compound of Example 2 (32.7 g, 107.9 mmol, 1 equiv.) and ethyl-6-hydroxyhexanoate (86.5 g, 539.6 mmol, 5 equiv.) in acetonitrile (20mL) was added 3N HCl (260 µl). All the reactants went into the solution after 15 min at room temperature. After the mixture was stirred over 24 hours, a small aliquot was taken for TLC and Mass Spec. to determine completion. Then, acetic anhydride (55.1 g, 539.6 mmol, 5 equiv.) and pyridine (42.7 g, 539.6 mmol, 5 equiv.) were added into the reaction to cap the excess hydroxyester-linker. The reaction was allowed to continue overnight.

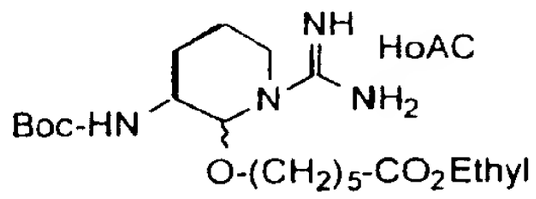
The residue was evaporated. The residue was taken up in ethyl acetate and washed with 1N HCl (1 X), water (1 X), saturated sodium bicarbonate (1 X), water (1 X) and dried (magnesium sulfate), filtered, and concentrated. The residue was purified by flash chromatography of silica gel, using 25-33% hexane/ethyl acetate gradient and afforded product 45.0 g (93.7% yield) as a viscous oil. R_f = 0.24 (50% hexanes/ethyl acetate)

MS: $(M + H^+)$ = 446.0, calculated (MW) = 445.2

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, 3H), 1.31-1.38 (m, 2H), 1.45 (s, 9H), 1.50-1.83 (m, 8H; aliph., b, g), 2.25-2.35 (m, 2H), 2.05-3.28 (m, 1.5H), 3.30-3.45 (m, 2H), 3.55-3.65 (br, .5H), 3.75-3.83 (br, 1H), 4.05-4.15 (m, 2H), 4.85 (d, 1H), 5.60 (s, 1H)

Example 4

Preparation of N-α-t-butoxycarbonyl-argininal (6-hexanoic acid ethyl ester) cyclol, acetate salt



To a solution of the compound of Example 3 (45.8 g, 103.0 mmol, 1 equiv.) in ethanol/water/acetic acid (4:1:1) (200mL) was added 10% palladium on carbon (9.2 g, 20% by weight). The mixture was hydrogenated at 40 psi for 16 h. The solution was filtered, and the filtrate was evaporated in vacuo. The residue was taken in water and washed with ether (3 X). The organic layers were re-extracted with water (1 X). Aqueous phases were combined and lyophilized to afford product 44.7 g (94.4% yield).

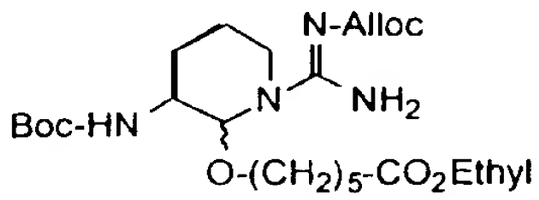
R_f = 0.18 (dichloromethane/methanol/conc. ammonium hydroxide; 25:5:1).

MS: $(M + H^+)$ = 401.0, calculated (MW) = 400.2

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, 3H), 1.31-1.38 (m, 2H), 1.45 (s, 9H), 1.50-1.83 (m, 8H; aliph., b, g), 2.25-2.35 (m, 2H), 3.05-3.28 (m, 1.5H), 3.32-3.48 (m, 2H), 3.55-3.61 (br, .5H), 3.63-3.73 (m, 1H), 4.08-4.15 (m, 2H), 4.89 (d, 1H), 5.14 (d, 1H)

Example 5

Preparation of N-α-t-butoxycarbonyl-N-omega-allyloxycarbonyl-argininal (6-hexanoic acid ethyl ester) cyclol



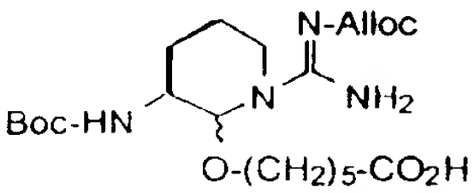
To a suspension of the compound of Example 4 (44.7 g, 97.3 mmol, 1 equiv.) in dichloromethane (292mL) at 0°C was added a solution of 1N sodium hydroxide (291.9mL, 291.9 mmol, 3 equiv.) portion-wise to maintain pH = 11-13. Allylchloroformate (15.3 g, 126.5 mmol, 1.3 equiv.) was added in three-portions into the reaction. After monitoring the reaction by TLC and MS for 1 h, the mixture was extracted with dichloromethane (3 X), dried (magnesium sulfate), filtered, and evaporated. The crude residue was purified immediately by flash chromatography of silica gel, using hexane and ethyl acetate as solvents. The product was obtained in 41.5 g (88.0% yield) as a viscous oil. Rf = 0.30 (50% hexanes/ethyl acetate)

MS: $(M + H^+) = 485.0$, calculated (MW) = 484.2

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24 (t, 3H), 1.31-1.38 (m, 2H), 1.45 (s, 9H), 1.50-1.83 (m, 8H;aliph., b, g), 2.25-2.33 (m, 2H), 2.90-3.12 (m, 1.5H), 3.30-3.45 (m, 2H), 3.55-3.65 (br, .5H), 3.75-3.83 (br, 1H), 4.05-4.15 (m, 2H), 4.57 (d, 2H), 4.83 (s, 1H), 5.25 (q, 2H), 5.90-6.05 (m, 1H)

Example 6

Preparation of N-α-t-butoxycarbonyl-N-δ-Alloc- N-α-t-butoxycarbonyl-N-omega-allyloxycarbonyl-argininal (6-hexanoic acid) cyclol



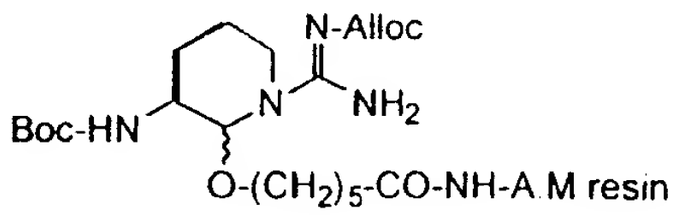
To a solution of the compound of Example 5 (40.3 g, 83.2 mmol) in ethanol (83.2mL) was added 3N lithium hydroxide (55.5mL, 166.4 mmol, 2 equiv.). Following stirring at room temperature for 2 1/2 h, the reaction was concentrated in vacuo. The residue was dissolved in water and washed with ether (3 X). The aqueous phase was acidified to pH = 2-3 with 1N HCl and extracted with dichloromethane. The solution was dried (magnesium sulfate), filtered, and evaporated to afford product 31.5 g (83.0% yield) as a white glassy foam. R_f = 0.33 (ethyl acetate).

MS: $(M + H^+)$ = 457.1, calculated (MW) = 456.2

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H), 1.52-1.87 (m, 10H; aliph., b, g), 2.32-2.43 (m, 2H), 3.05-3.28 (m, 1.5H), 3.37-3.55 (m, 2H), 3.83 (s, 1H), 4.57-4.65 (m, 2H), 4.88 (d, 1H), 5.31 (q, 2H), 5.53 (s, 1H), 5.90-6.05 (m, 1H)

Example 7

Preparation of N-α-t-butoxycarbonyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol

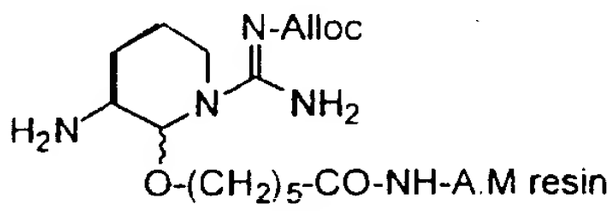


To amino methylated polystyrene resin (22.6 g, 26.2 mmol, 1 equiv.), the compound of Example 6 (15.5 g, 34.0 mmol, 1.3 equiv.), and PyBOP (17.7 g, 34.0 mmol, 1.3 equiv.) in dimethylformamide (214mL) at room temperature was added, followed by diisopropylethylamine (4.4 g, 34.0 mmol, 1.3 equiv.). The mixture was allowed to stir slowly in a round bottom flask overnight. The resin was washed with copious amounts of dichloromethane and methanol. The resin was dried under vacuum (a 3 mg sample of dried resin was taken out for Kaiser Test) and acetylated with dimethylformamide/acetic anhydride/triethylamine (8:1:1) for 30 min at ambient temperature. Once again, the resin

was washed successively with organic solvents (dichloromethane and methanol), and dried under vacuum to afford product 40.8 g (92.0% yield by weight). Kaiser Test (O.D: 99% coupled); Resin substitution (approx. 0.75 mmol/g)

Example 8

Preparation of N^{δ}-Alloc-Arginylcyclo-O-6-hydroxylhexanoyl-amino methylated polystyrene



A solution of DCM/TFA/Thioanisole (6:3:1) (5 mL) was added to the resin of Example 7 (200 mg, 0.15 mmol, 1 equiv.). The mixture was allowed to stir slowly in a round bottom flask at ambient temperature for 25 minutes with nitrogen gas bubbled in. The reactants were drained from the resin and the deprotected resin was washed successively with DCM (2 X), DCM/DIEA (2 X), DCM (2 X), and alternatively with organic solvents (methylene chloride and methanol). The resulting title resin was dried under vacuum. Kaiser Test (solution and bead; dark blue); Resin substitution (0.81 mmol)

Example 9

Solid-Phase Synthesis of isobutyloxycarbonyl-D-seryl-L-alanyl-argininal (compound 1)

This Example describes a general procedure for solid-phase syntheses of an arginine aldehyde having urokinase inhibitory activity, using the resin of Example 7. The procedures of this Example were also followed to synthesize a library of compounds, wherein each member comprised an arginine aldehyde but differed from the other members of the library in one or more other substituents.

Step 1: Preparation of N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol

To a 60mL solid phase reaction vessel was added 2.0 g of the compound of Example 7 (0.6-0.7 meq/g substitution), and a mixture of dichloromethane (12mL), trifluoroacetic acid (6mL), and thioanisole (2mL). Nitrogen gas was bubbled for 15 min. The reactants were drained from the resin and the resin was washed successively with dichloromethane(2X20mL), diisopropylethylamine (20mL), dichloromethane (2X20mL), diisopropylethylamine (20mL), dichloromethane (2X20mL), and diethyl ether (2X20mL). The title compound was stored under vacuum. A ninhydrin test of the resin showed a dark blue color characteristic of the free amine produced by the removal of the t-butoxycarbonyl group.

Step 2: Preparation of N-α-Fmoc-alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol

The compound of Step 1 (2.1 g) was placed in a solid phase reaction vessel to which were added Fmoc-alanine (1g, 3.2 mmol), 1-hydroxybenzotriazole (0.5g, 3.2 mmol), TBTU (1.024g, 3.2 mmol) and diisopropylethylamine (600 µL, 3.4 mmol) in dimethylformamide (15-20mL). Nitrogen gas was bubbled through the reactor at room temperature for 2 h. The reagents were drained from the resin and the resin was washed successively with dimethylformamide (2X20mL), dichloromethane (2X20mL), dimethylformamide (2X20mL), dichloromethane (2X20mL), and diethyl ether (2X20mL). The resin was vacuum dried and a small aliquot was taken for ninhydrin colorimetric analysis, which showed a 99.5% coupling efficiency in the production of the title compound.

Step 3: Preparation of alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol

The compound of Step 2 was treated with 50% piperidine in dimethylformamide (20mL) for 30 min at room temperature with nitrogen gas agitation. The resin was washed as above and vacuum dried to give the title compound. A ninhydrin assay on a small aliquot gave dark blue resin and solution showing a high yield for the deprotection.

Step 4: N-α-Fmoc-D-seryl(O-t-butyl)-alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol

The compound of Step 3 (100 mg) was placed in a reaction vessel, such as a Kan™ reaction vessel (IRORI, San Diego, CA). The reaction vessel was placed in a 20mL vial containing dimethylformamide (4mL), N-α-Fmoc-D-serine(O-t-butyl) (184mg, 0.48mmol), 1-hydroxybenzotriazole (73mg, 0.48mmol), TBTU (154mg, 0.48mmol), and diisopropylethylamine (84µl, 0.48mmol). The reaction vessel was agitated for 3 h on a shaker table. The reaction vessel was drained, washed successively with dimethylformamide (2X3mL), dichloromethane(2X3mL), dimethylformamide(2X3mL), dichloromethane(2X3mL), isopropanol(2X3mL), dichloromethane(2X3mL), isopropanol(2X3mL), and diethyl ether(2X3mL). The resin was dried under vacuum to give the title compound.

Step 5: D-seryl(O-t-butyl)-alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol

The reaction vessel containing the compound of Step 4 was treated with 50% piperidine in dimethylformamide (5mL) in a 20mL vial for 45 min at room temperature while agitated on a shaker table. The resin was washed as above and vacuum dried to give the compound of Step 5. A ninhydrin assay on a small aliquot gave a dark blue resin and solution indicating a high yield for the deprotection.

Step 6: carbamate analogs of D-seryl(O-t-butyl)-alanyl-N-omega-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol

The reaction vessel containing the compounds of Step 5 was placed in a 20mL vial with 0.12M isobutylchloroformate, in dimethylformamide (5-10mL). Diisopropylethylamine (105-210µl, 0.6-1.2 mmol) was added, and the vial was shaken for 2.5 h. The reaction vessel was drained and washed successively with dimethylformamide (2X3mL), dichloromethane(2X3mL), dimethylformamide(2X3mL), dichloromethane(2X3mL), isopropanol(2X3mL), dichloromethane(2X3mL), isopropanol(2X3mL), and diethyl ether(2X3mL). The reaction vessel containing isobutyloxycarbonyl-D-seryl(O-t-butyl)-alanyl-N-d-allyloxycarbonyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol was vacuum dried.

When the procedures of Example 9 were followed to make a library of related analogs, reaction vessels containing related resin-bound analogs were combined in the 20 ml vial in Step 6, with various individual chloroformates added, and treated in parallel for the remainder of Steps 7 and 8.

Step 7: carbamate analogs of D-seryl(O-t-butyl)-alanyl- argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol

Removal of the allyloxy protecting group from the product of Step 5 was accomplished by placing a collection of 38 reaction vessels, including the reaction of interest, in a 250mL polypropylene bottle and adding a mixture of methylsulfoxide (10mL), tetrahydrofuran (10mL), 1N HCl (2.5mL), and morpholine (25mL). Tetrakis triphenylphosphine palladium (0.87g) was then added, and the bottle was shaken for 4 h at room temperature. The reaction vessel, including the one containing isobutyloxycarbonyl-D-seryl(O-t-butyl)-alanyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol, were drained, washed successively with dimethylformamide

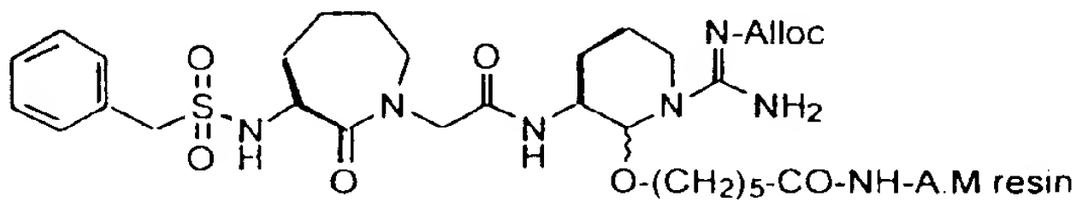
(2X3mL), dichloromethane(2X3mL), dimethylformamide(2X3mL), dichloromethane(2X3mL), isopropanol(2X3mL), dichloromethane(2X3mL), isopropanol(2X3mL), and diethyl ether(2X3mL), and vacuum dried to give the title compound.

Step 8: isobutyloxycarbonyl-D-seryl-alanyl-argininal

The reaction vessel containing isobutyloxycarbonyl-D-seryl(O-t-butyl)-alanyl-argininal (6-hexanoyl-aminomethylated polystyrene resin) cyclol was emptied into a Whatman polypropylene mini-column containing trifluoroacetic acid/dichloromethane/water (1.5mL of a 6:3:1 mixture). The column was shaken for 4 h at room temperature. The reaction solution was drained into a test tube and the resin was washed with dichloromethane and water. The wash mixtures and the reaction mixtures were collected in the same test tube, and the mixture was agitated, then allowed to separate into two phases. The water layer was removed and filtered. The title compound was purified by semipreparative reverse-phase HPLC, lyophilized, and weighed, and analyzed by HPLC and mass spectrometry. The title compound was shown to have urokinase inhibitory activity in *in vitro* assays.

Example 10

Preparation of Benzylsulfonamido-7Lactam-Gly-N^{δ}-Alloc-Arginylcyclo-O-6-hydroxylhexanoyl-amino methylated polystyrene

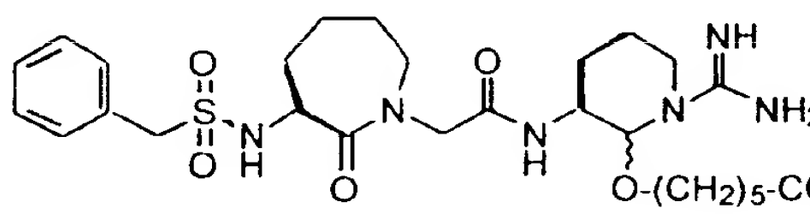


To a mixture of the resin of Example 8 (180 mg, 0.15 mmol, 1 equiv.), Benzylsulfonamido-7Lactam-Gly-OH (65 mg, 0.19 mmol, 1.3 equiv.; see examples 27-31 of US 5,703,208 for synthesis), and BOP (84.0 mg, 0.19 mmol, 1.3 equiv.) in DMF (2

mL) was added DIEA (24.6 mg, 0.19 mmol, 1.3 equiv.). The mixture was allowed to shake in a Whatman polypropylene mini-column at room temperature overnight. The resin was washed with organic solvents (methylene chloride and methanol) and dried. Kaiser Test (O.D: 99.9%; Solution and Bead: clear). Based on theoretical yield (230 mg), Resin substitution (0.636 mmol/g).

Example 11

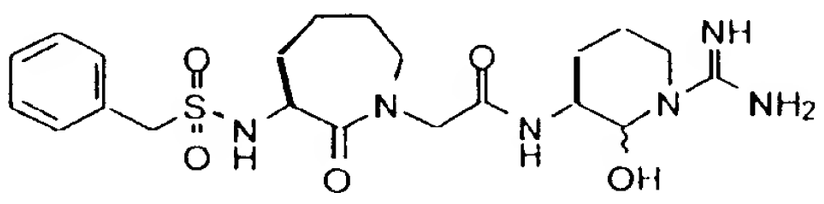
Preparation of Benzylsulfonamido-7Lactam-Gly-Arginylcyclo-O-6-hydroxyhexanoyl-amino methylated polystyrene



To a solution of THF/DMSO/.5N HCl/Morpholine (2:2:1:5) (1.5 mL) was added the resin of Example 10 (30 mg, 0.019 mmol, 1 equiv.) and $(Ph_3P)_4$ Pd (10.2 mg, 34% by weight). The mixture was allowed to shake in a Whatman polypropylene mini-column at room temperature for 3 hours. The resin was washed with organic solvents (methylene chloride and methanol) and dried. Based on theoretical (28.3 mg), Resin substitution (0.672 mmol/g).

Example 12

Preparation of Benzylsulfonamido-7Lactam-Gly-Arginal



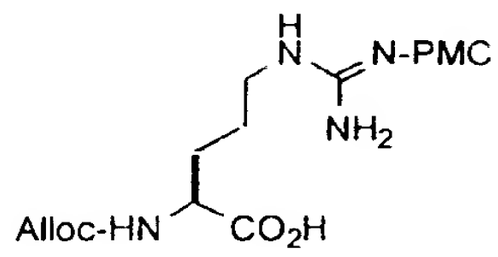
A solution of TFA/H_2O/DCM (5:1:4) (1.5 mL) was added to the resin of Example 11 (20 mg, 0.013 mmol, 1 equiv.) in a Whatman polypropylene mini-column. The mixture was allowed to shake by mechanical shaker for 2 hours at ambient temperature. The filtrate was purified with a Sepak column, using a H_2O, 0.1% acetonitrile; 10,0 to 9,1 gradient to afford 2.6 g (42.0% yield) of title product as a white-powder (fluffy).

MS (M + H) = 481.0, calculated (MW) = 479.2

HPLC retention, 13.1, 13.7, and 14.0 min.(2 cyclol and hydrate forms of the arginal derivative); C_{18} 5 - 50% MeCN over 25 minutes, 1.0 mL/min. The title compound was shown to have thrombin inhibitory activity in *in vitro* assays.

Example 13

Preparation of N-α-Alloc-N^{δ}-PMC-Arginine:



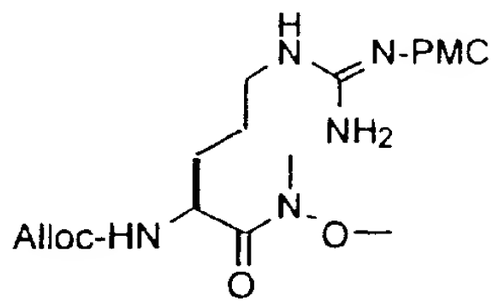
To a suspension of N^{d}-PMC-Arginine (25.0 g, 56.7 mmol,) in tetrahydrofuran (250 mL) at O°C was added a solution of 1N NaOH (62.4 mL, 62.4 mmol, 1.1 equiv.) portionwise to maintain pH = 8-9. Allyl chloroformate (7.52 g, 62.4 mmol, 1.1 equiv.) was slowly added over 10 minutes. After the addition was completed, the reaction was allowed to stir at ambient temperature for 2 1/2 hours. The solvent was removed in vacuo. The residue was partitioned between ethyl acetate (100 mL) and water (150 mL). The aqueous phase was acidified to pH = 2-3 with 1N HCl and extracted with ethyl acetate (2 X 150 mL). The combined extracts were washed with 1N HCl (1 X 200 mL), H_2O (1 X 200 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo overnight to afford 29.7g (100% yield) of product as a white foam, judged pure by TLC (silica; 27 MC/ 3 M/ 1 A) Rf = 0.3.

MS (M + H) = 525.2, calculated (MW) = 524.2

NMR (400 MHz, $CDCl_3$): δ 1.30 (s, 6H), 1.55-1.95 (m, 6H), 2.12 (s, 3H), 2.55 (d, 6H), 2.60-2.68 (m, 2H), 3.17-3.30 (br, 2H), 4.25-4.37 (br, 1H), 4.59 (d, 2H), 5.27 (q, 2H), 5.85-5.97 (m, 1H), 6.07-6.15 (br, 1H).

Example 14

Preparation of N-α-Alloc-N^{δ}-PMC-Arginine-N-methoxy-N-methylamide



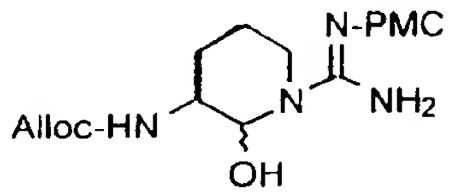
To a solution of the compound of Example 13 (29.7 g, 56.7 mmol,), N,O-dimethylhydroxylamine hydrochloride (11.1 g, 113.4 mmol, 2.0 equiv.), EDC (14.1 g, 73.7 mmol, 1.3 equiv.), and HOBT (10.0 g, 73.7 mmol, 1.3 equiv.) in acetonitrile (150 mL) was added N-methylmorpholine (8.6 g, 9.4 mL, 85.1 mmol, 1.5 equiv.). The reaction was allowed to stir at ambient temperature for 18 hours. The solvent was evaporated under vacuum. The residue was partitioned between ethyl acetate (150 mL) and water (250 mL). The aqueous layer was re-extracted with ethyl acetate (2 X 150 mL). The combined organic phases were washed with 1N HCl (1 X 200 mL), H_2O (1 X 200 mL), saturated $NaHCO_3$ (1 X 200 mL), and H_2O (1 X 200 mL). The product was dried over $MgSO_4$ and filtered. Evaporation of the filtrate gave 32.1 g (100% yield) of product as a foam. TLC (silica; 9 methylene chloride / 1 ethanol) Rf = 0.53.

MS (M + H) = 568.1, calculated (MW) = 567.2

NMR (400 MHz, $CDCl_3$): δ 1.30 (s, 6H), 1.55-1.85 (m, 6H), 2.07 (s, 3H), 2.45-2.67 (m, 8H), 3.19 (s, 3H), 3.28-3.40 (br, 2H), 3.75 (s, 3H), 4.50 (d, 2H), 4.61-4.75 (br, 1H), 5.23 (q, 2H), 5.77-5.90 (m, 1H).

Example 15

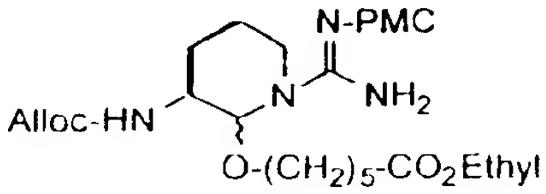
Preparation of N-α-Alloc-N$^{\delta}$-PMC- Argin-al



A solution of the compound of Example 14 (32.0 g, 56.4 mmol,) in anhydrous tetrahydrofuran (500 mL) in a 2 Liter 3 neck round bottom flask under nitrogen atmosphere was added 1N LAH/THF (85.0 mL, 85.0 mmol, 1.5 equiv.) drop wise over 1 hour. The reaction was stirred at -78°C for 2 hours and then allowed to warm up to 0°C for 30 minutes. A thick slurry was observed. The reaction mixture was quenched slowly with 2M $KHSO_4$ (100 mL) at -78°C. The precipitate was filtered out and washed with tetrahydrofuran (200 mL). The combined filtrate was concentrated in vacuo. The crude residue was partitioned between ethyl acetate (200 mL) and H_2O (200 mL). The organic layers were washed with saturated $NaHCO_3$ (2 X 200 mL), and brine (1 X 200 mL). The product was dried over $MgSO_4$ and filtered. Evaporation of the filtrate gave 28.7 g (100% yield) of product as a white foam. TLC (silica; 1 hexane / 1 ethyl acetate) Rf = .1
MS (M + H) = 509.1, calculated (MW) = 508.2
NMR (400 MHz, $CDCl_3$): δ 1.30 (s, 6H), 1.51-2.50 (m, 6H), 2.03-2.10 (m, 3H), 2.45-2.67 (m, 8H), 3.07-3.26 (m, 1.5H), 3.57-3.67 (m, .5H), 4.52 (d, 2H), 5.23 (q, 2H), 5.56 (d, 1H), 5.60-5.67 (br, 1H), 5.80-5.95 (m, 1H).

Example 16

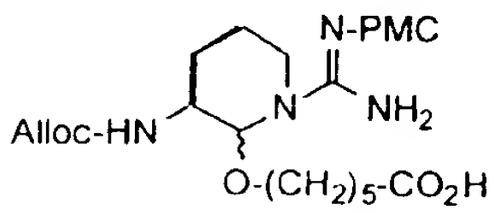
Preparation of N-α-Alloc-N$^{\delta}$-PMC-Arginylcyclo-O-Ethyl-6-hydroxylhexanoate



To a stirred solution of the compound of Example 15 (28.7 g, 56.5 mmol,) and ethyl-6-hydroxyhexanoate (45.3 g, 283 mmol, 5 equiv.) in acetonitrile (200 mL) was added 3N HCl (130 µL). All the reactants went into solution over 15 minutes at room temperature. After the mixture was stirred for 24 hours (a 40 µL aliquot was taken for TLC and Mass Spec. for the determination of the completion), acetic anhydride (22.4 g, 283 mmol, 5 equiv.) and pyridine (22.4 g, 283 mmol, 5 equiv.) were added into the reaction mixture to cap the excess hydroxy-linker. The reaction was allowed to continue overnight. The solution was evaporated. The crude product was taken up in ethyl acetate and washed with 1N HCl (1 X), water (1 X), saturated $NaHCO_3$ (1 X), water (1 X), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography on silica gel, using a hexane, ethyl acetate: 4,1 to 2,1 gradient and afforded product 22.2 g (61% yield) as a viscous oil. TLC (silica; hexane, ethyl acetate: 1,1) Rf = 0.13
MS (M + H) = 651.1, calculated (MW) = 650.3
NMR (400 MHz, $CDCl_3$): δ 1.30 (s, 9H), 1.36-1.75 (m, 10H; aliph., b, g), 1.75-1.85 (m, 2H), 2.10 (s, 3H), 2.22-2.31 (m, 2H), 2.50-2.69 (m, 8H), 2.93-3.15 (m, .5H), 3.25-3.43 (m, 2H), 3.57-3.72 (m, 1.5H), 4.09-4.16 (m, 2H), 4.45-4.52 (br, 1H), 4.53-4.60 (m, 2H), 5.03 (d, 1H), 5.15-5.35 (m, 2H), 5.43-5.50 (br, 1H), 5.80-5.98 (m, 1H), 6.30-6.42 (m, 2H)

Example 17

Preparation of N-α-Alloc-N^{δ}-PMC- Arginylcyclo-O-6-hydroxylhexanoic acid



To a solution of the compound of Example 16 (22.2 g, 34.2 mmol, 1 equiv.) in ethanol (34.2 mL) was added 3N LiOH (68.4 mmol, 22.8 mL, 2 equiv.). The reaction was stirred at room temperature for 4 hours and concentrated under vacuum. The residue was dissolved in H_2O (50 mL) and washed with ether (2 X 50 mL). The aqueous layer was

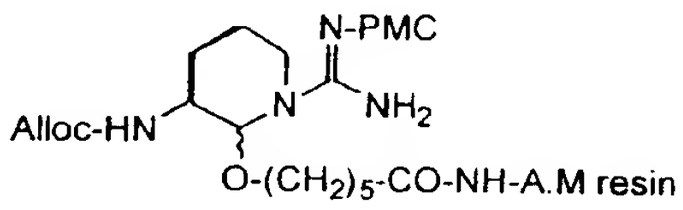
acidified to pH = 2-3 by 1N HCl and extracted with methylene chloride (3 X 100 mL). The solution was dried over $MgSO_4$, filtered, and evaporated to afford product 16.73 g (79% yield) as a white foam. TLC (silica; ethyl acetate) Rf = 0.49

MS (M + H) = 623.0, calculated (MW) = 622.3

NMR (400 MHz, $CDCl_3$): δ 1.30 (s, 6H), 1.45-1.75 (m, 10H; aliph., b, g), 1.75-1.85 (m, 2H), 2.11 (s, 3H), 2.30-2.40 (m, 2H), 2.50-2.69 (m, 8H), 2.93-3.13 (m, .5H), 3.25-3.50 (m, 2H), 4.55-4.72 (m, 1.5H), 4.45-4.52 (br, 1H), 4.53-4.60 (m, 2H), 5.06 (d, 1H), 5.15-5.35 (m, 2H), 5.43-5.50 (br, 1H), 5.80-5.98 (m, 1H), 6.30-6.45 (m, 2H).

Example 18

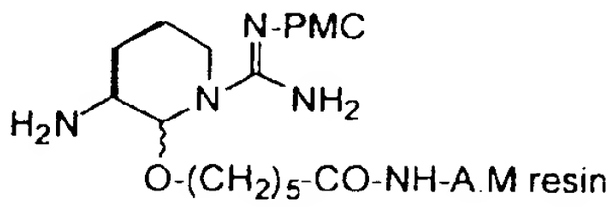
Preparation of N-α-Alloc-N^{δ}-PMC- Arginylcyclo-O-6-hydroxylhexanoyl-amino methylated polystyrene resin



To amino methylated polystyrene resin (16.0 g, 18.6 mmol, 1 equiv.), the compound of Example 17 (15.0 g, 24.1 mmol, 1.3 equiv.), PyBOP (12.5 g, 24.1 mmol, 1.3 equiv.) in DMF (193 mL) at room temperature was added DIEA (3.1 g, 24.1 mmol, 1.3 equiv.). The mixture was allowed to stir slowly in a round bottom flask overnight. The resin was washed alternatively and excessively with methylene chloride and methanol. The resin was dried under vacuum (a 2 mg portion of the resin was taken for a Kaiser Test), and then acetylated with DMF/acetic anhydride/Et_3N (8:1:1) for 30 minutes at ambient temperature. Once again, the resin was washed with organic solvent (methylene chloride and methanol) and dried under vacuum to afford product 26.9 g (98.5% by weight). Kaiser Test (O.D; 97.7% coupled); Resin substitution (0.67 mmol/g).

Example 19

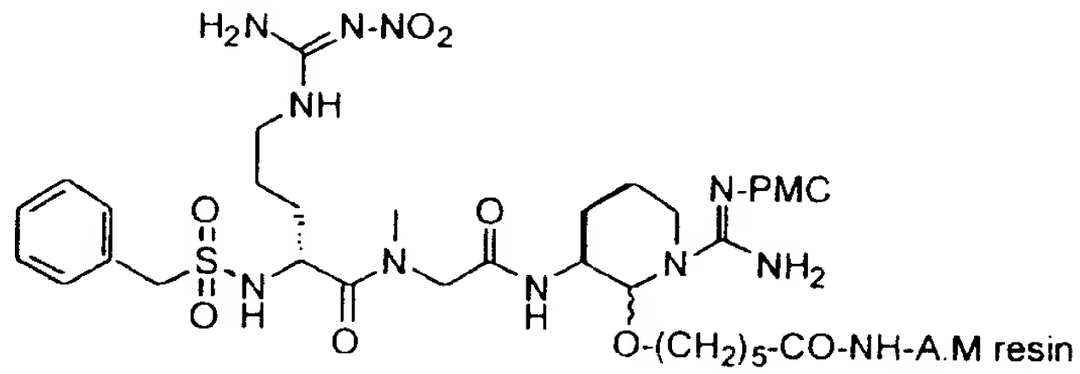
Preparation of N^d-PMC-Arginylcyclo-O-6-hydroxyhexanoyl-amino methylated polystyrene resin



A solution of THF/DMSO/0.5N HCl/Morpholine (2:2:1:5) (1.5 mL) was added to the resin of Example 18 (50 mg, 0.03 mmol, 1 equiv.), and $(Ph_3P)_4$ Pd (26 mg, 0.02 mmol, 0.65 equiv.). The mixture was allowed to shake in a Whatman polypropylene mini-column at ambient temperature for 2 hours. The deprotected resin was then washed with organic solvents (methylene chloride and methanol) and dried. Kaiser Test (solution and bead; dark blue), Resin substitution (0.71 mmol/g).

Example 20

Preparation of Benzylsulfonamido-dArg(NO_2)-Sarcosine-Gly- N^{δ}-PMC-Arginylcyclo-O-6-hydroxyhexanoyl amino methylated polystyrene resin

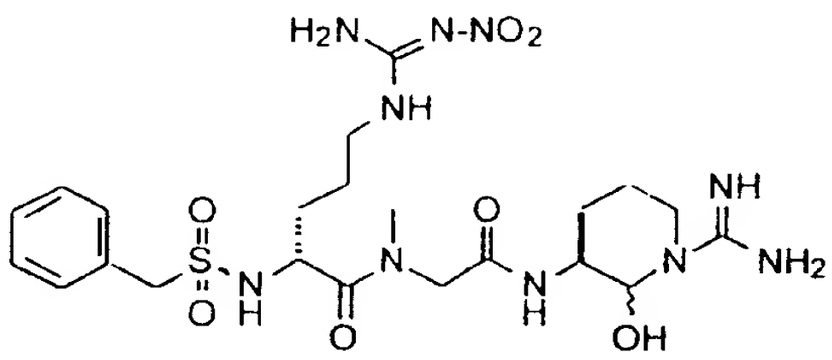


To a mixture of the resin of Example 19 (35 mg, 0.025 mmol, 1 equiv.), Benzylsulfonamido-dArg(NO_2)-Sarcosine-Gly-OH (45 mg, 0.10 mmol, 4 equiv.; see Examples 11-13 of US 5,696,231 for preparation), PyBOP (53 mg, .10 mmol, 4 equiv.) in DMF (0.60 mL) was added DIEA (13 mg, 0.1 mmol, 4 equiv.). The mixture was allowed to shake in a Whatman mini-column overnight. Resin was washed with organic solvent

(methylene chloride and methanol) and dried. Kaiser Test (solution and bead; clear). Based on theoretical yield (45.9 mg), Resin substitution (0.545 mmol/g).

Example 21

Preparation of Benzylsulfonamido-dArg(NO_2)-Sarcosine-Gly-Arginal



A solution of TFA/H_2O/DCM (5:1:4) (1.5 mL) was added to the resin of Example 20 (45.9 mg, 0.025 mmol) in a Whatman mini-column tube. The mixture was allowed to shake mechanically for 4 hours at room temperature. The filtrate was purified using a Sepak column. Fractions were collected, lyophilized, and analyzed by HPLC and Mass Spec to afford title product 0.8 mg (5.5% yield) as white powder.

MS (M + H) = 585.0, calculated (MW) = 584.2

HPLC retention, 7.3 min.; C_{18}, 5 - 20% CH_3CN over 25 minutes, 1.0 mL/min. The title compound was shown to have Factor Xa inhibitory activity in *in vitro* assays.

WHAT IS CLAIMED IS:

1. A method for making a derivatized solid support represented by formula (I):

(I) R1-O-X-$(CH_2)_n$-Y-CO-NH-SS

wherein:

(a) R1 is derived from R1', a moiety having a cyclic amino aldehyde or derivative thereof at its carboxy-terminus, wherein the moiety R1' optionally comprises a protecting group at one or more positions thereof;

(b) n is an integer from about 1 to about 15, provided that if both X and Y are bonds, n is at least two;

(c) SS is a solid support; and

(d) X and Y are independently a bond or -$[Z]_p$-, wherein: p is an integer between 1 and 5, provided that the combination of X, Y and -$(CH_2)_n$- represents a chain equivalent in length to a linear chain of one to about fifteen carbon atoms; Z is -CH_2CH_2O-, or -C(A)(B)-, wherein A and B may vary in each occurrence of Z, and are independently selected from the group consisting of hydrogen, alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbons, and wherein in combination, A and B may form a five to seven membered ring;

said method comprising the steps of

(a) (i) contacting R1' with a linker of formula OH-X-$(CH_2)_n$-Y-CO_2-R2, wherein R2 is selected from the group consisting of -NH-SS, if the linker is first reacted with a solid support, or H, lower alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbon atoms, and aralkyl of about six to about fifteen carbon atoms, under acid-catalyzed conditions permitting reaction of a carbonyl or nascent carbonyl of R1' with the hydroxy of the linker to form R1-O-X-$(CH_2)_n$-Y-CO_2-R2;

(ii) recovering R1-O-X-$(CH_2)_n$-Y-CO_2-R2 and, when R2 is not -NH-SS, or -H, subjecting the R2 group to hydrolysis to produce a carboxylic acid of formula R1-O-X-$(CH_2)_n$-Y-CO-OH;

(iii) contacting R1-O-X-$(CH_2)_n$-Y-CO-OH with a NH_2-SS, unless the linker was previously reacted with a solid support, under conditions permitting dehydration of the carboxylic acid and amide bond formation between the carboxylic acid and the NH_2-SS to form R1-O-X-$(CH_2)_n$-Y-CO-NH-SS, and

(iv) recovering the R1-O-X-$(CH_2)_n$-Y-CO-NH-SS solid support thus formed.

2. The method according to claim 1 wherein n is about 2-7.

3. The method according to claim 2 wherein n is about 5.

4. The method according to claim 1 wherein the combination of X, Y and -$(CH_2)_n$- represents a chain equivalent in length to a linear chain of about five carbon atoms.

5. The method according to claim 4, wherein n is about 5.

6. The method according to claim 1 wherein R1' is an arginine aldehyde, an arginine aldehyde derivative, or a derivative of aspartic acid, homoserine, or homocysteine.

7. The method according to claim 6, wherein R1' is incorporated in a peptidyl composition.

8. The method according to claim 6 wherein a ω-side chain of said cyclic amino aldehyde or derivative thereof is protected with a protecting group.

9. The method according to claim 8 wherein said protecting group is selected from the group consisting of a Boc, Alloc, PMC, and Fmoc.

10. The method according to claim 1 wherein an alpha or omega heteroatom of R1' is blocked with a first or a second protecting group, PG1 and PG2, respectively, prior to step (i), or wherein the alpha nitrogen of R1 is blocked with PG1 prior to step (i) and the omega nitrogen of R1 is blocked with PG2 after step (ii).

11. The method according to claim 10 wherein PG1 and PG2 are protecting groups selected to be orthogonal.

12. The method according to claim 11 wherein PG1 and PG2 are selected from the following combinations of protecting groups: Boc with Fmoc, Alloc, or Teoc; Fmoc with Alloc, Teoc, or PMC; and Alloc with Teoc or PMC.

13. The method according to claim 1 wherein said NH_2-SS is selected from the group consisting of amino methylated polystyrene resin, benzhydrylamine resin, and 4-methylbenzhydrylamine resin.

14. The method according to claim 1 wherein said NH_2-SS is selected from the group consisting of amino methylated polystyrene resin, benzhydrylamine resin, and 4-methylbenzhydrylamine resin, and wherein n is about 5.

15. The method according to claim 14 wherein the linker is HO-$(CH_2)_5$-CO_2-ethyl, and NH2-SS is amino methylated polystyrene resin.

16. The method according to claim 1 wherein R2 is selected from the group consisting of alkyl, alkenyl, and aralkyl.

17. The method according to claim 16 wherein R2 is ethyl.

18. The method according to claim 1 wherein said linker is OH-$(CH_2)_5$-CO_2-ethyl.

19. The method according to claim 1 for solid-phase peptide synthesis which comprises the step of linking to the group R1 one or more amino acids, amino acid analogs, peptides or peptide analogs to form a peptide or peptidomimetic chain of amino acids or amino acid derivatives comprising the cyclic aldehyde or nascent aldehyde R1 at the carboxy terminus thereof to form a product represented by formula (III):

(III) $(res)_t$-R1-O-X-$(CH_2)_n$-Y-CO-NH-SS,

wherein the moiety $(res)_t$- represents a peptide or peptide analog comprising "t" residues, wherein "t" is an integer between about one and fifty.

20. The method according to claim 19 wherein "t" is an integer between about one and about 10.

21. The method according to claim 20 for synthesis of a protease inhibitor.

22. The method according to claim 21 wherein said inhibitor is a serine, cysteine, or aspartyl protease inhibitor.

23. The method according to claim 19 comprising the further step of cleaving the moiety $(res)_t$-R1 from the derivatized resin.

24. The method according to claim 23 wherein said cleaving comprises treatment of the derivatized resin represented by formula (III) with a mixture of TFA:DCM:H_2O in a ratio of 6:3:1, and wherein said cleaving is carried out for about two hours.

25. A method for making a solid support represented by the formula:

$R3[-O-X-(CH_2)_m-Y-CO-NH-SS]_2$ (II);

wherein:

(a) R3 is a moiety derived from R3' having a linear chain amino aldehyde, or a derivative thereof, at its carboxy-terminus, wherein the moiety R3' optionally comprises a protecting group at one or more positions;

(b) m is an integer of between about 1 and 15, provided that m is at least two if both X and Y are bonds;

(c) SS is a solid support; and

(d) X and Y are independently a bond or $-[Z]_p-$, wherein p is an integer between 1 and 5, provided that the combination of X, Y and $-(CH_2)_m-$ represents a chain equivalent in length to linear chain of about two to fifteen, and preferably about five carbon atoms; Z is $-CH_2CH_2O-$, or -C(A)(B)-, wherein A and B may vary in each occurrence of Z, and are independently selected from the group consisting of hydrogen, alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbons, and wherein in combination, A and B may form a five to seven membered ring;

said method comprising the steps of:

(i) contacting the R3' aldehyde with at least a two molar excess, as compared to R3, of a linker of formula:

$$OH-X-(CH_2)_m-Y-CO_2-R2$$

wherein R2 is selected from the group consisting of -NH-SS, if the linker has previously been reacted with a solid support, or -H, alkyl of one to about six carbon atoms, alkenyl of about three to about ten carbon atoms, aryl of about six to about ten carbon atoms, and aralkyl of about six to about fifteen carbon atoms, under acid catalyzed conditions permitting reaction of the carbonyl of R3' with the hydroxyl of the linker, to form:

$$R3[-O-X-(CH_2)_m-Y-CO_2-R2]_2,$$

(ii) recovering the product from (i) and, when R2 is not -NH-SS or -H, hydrolyzing R2 to provide a carboxylic acid,

(iii) contacting the carboxylic acid of (ii) with NH_2-SS, unless the linker was previously reacted with the solid support, under conditions permitting dehydration of the carboxylic acid and amide bond formation between the carboxylic acid and the NH_2-SS to form a solid support of formula:

$$R3[-O-X-(CH_2)_m-Y-CO-NH-SS]_2 \text{ (II); and}$$

(iv) recovering the solid support thus formed.

26. The method according to claim 25 wherein m is about 2-7.

27. The method according to claim 26 wherein m is about 5.

28. The method according to claim 25 wherein the combination of X, Y and $-(CH_2)_m-$ represents a chain equivalent in length to a linear chain of about five carbon atoms.

29. The method according to claim 28, wherein m is about 5.

30. The method according to claim 25 for solid-phase peptide synthesis which comprises the step of linking to the group R3 one or more amino acids, amino acid analogs, peptides or peptide analogs to form a peptide or peptidomimetic chain of amino acids or amino acid derivatives comprising the cyclic aldehyde or nascent aldehyde R1 at the carboxy terminus thereof to form a product represented by formula (IV):

(IV) $(res)_t$-R3(-O-X-$(CH_2)_m$-Y-CO-NH-SS)$_2$,

wherein the moiety $(res)_t$- represents a peptide or peptide analog comprising "t" residues, wherein "t" is an integer between about one and fifty.

31. The method according to claim 30 wherein "t" is an integer between about one and about 10.

32. The method according to claim 31 for synthesis of a protease inhibitor.

33. The method according to claim 32 wherein said inhibitor is a serine, cysteine, or aspartyl protease inhibitors.

34. The method according to claim 30 wherein an alpha or reactive side chain heteroatom of R3 is blocked with a first or a second protecting group, PG1 and PG2, respectively, prior to step (i), or wherein the alpha nitrogen of R3 is blocked with PG1

prior to step (i) and a reactive side chain heteroatom of R3 is blocked with PG2 after step (ii).

35. The method according to claim 34 wherein PG1 and PG2 are protecting groups selected to be orthogonal.

36. The method according to claim 35 wherein PG1 and PG2 are selected from the following combinations of protecting groups: Boc with Fmoc, Alloc, or Teoc; Fmoc with Alloc, Teoc, or PMC; and Alloc with Teoc or PMC.

37. The method according to claim 30 comprising the further step of cleaving the moiety $(res)_t$-R3 from the derivatized resin.

38. The method according to claim 37 wherein said cleaving comprises treatment of the derivatized resin represented by formula (IV) with a mixture of TFA:DCM:H_2O in a ratio of 6:3:1, and wherein said cleaving is carried out for about two hours.

39. The method according to claim 25 wherein said NH_2-SS is selected from the group consisting of amino methylated polystyrene resin, benzhydrylamine resin, and 4-methylbenzhydrylamine resin.

40. The method according to claim 25 wherein R2 is selected from the group consisting of alkyl, alkenyl, and aralkyl.

41. The method according to claim 40 wherein R2 is ethyl.

42. The method according to claim 25 wherein said linker is OH-$(CH_2)_5$-CO_2-ethyl.

43. The method according to claim 1 for preparing a combinatorial peptide library comprising an aldehyde carboxy-terminal residue linked to a solid support which comprises the steps of:

(a) in each of a series of separate containers or reaction vessels, contacting a solid support represented by the formula (I):

(I) R1-O-X-$(CH_2)_n$-Y-CO-NH-SS, wherein the moiety R1 represents a P1 residue,

with different amino acid residues or amino acid analog residues under conditions permitting formation of a peptide bond so as to produce a series of P_2 residues, same or different, in each of said series of separate containers or reaction vessels; and

(b) repeating step (a) as many times as required to generate a peptide or peptide analog of the desired number of residues, with appropriate intermediate steps of protection and deprotection of reactive groups present on the growing peptide or peptide analog chain.

44. The method according to claim 25 for preparing a combinatorial peptide library comprising an aldehyde carboxy-terminal residue linked to a solid support which comprises the steps of:

(a) in each of a series of separate containers or reaction vessels, contacting a solid support represented by the formula (II):

(II) R3[-O-X-$(CH_2)_m$-Y-CO-NH-SS]$_2$, wherein the moiety R3 represents a P1 residue,

with different amino acid residues or amino acid analog residues under conditions permitting formation of a peptide bond so as to produce a series of P_2 residues, same or different, in each of said series of separate containers or reaction vessels; and

(b) repeating step (a) as many times as required to generate a peptide or peptide analog of the desired number of residues, with appropriate intermediate steps of

protection and deprotection of reactive groups present on the growing peptide or peptide analog chain.

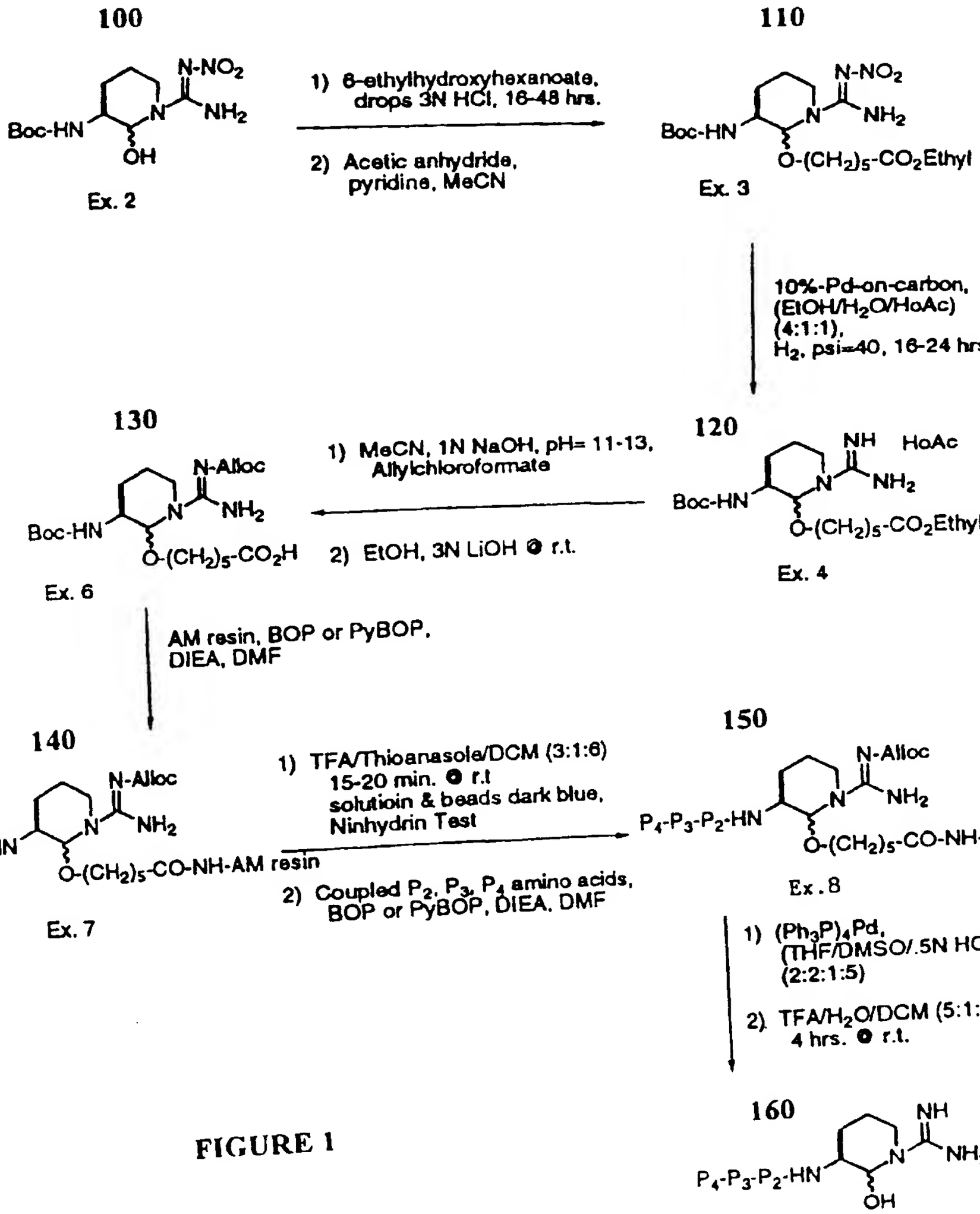

100
N-NO2
NH2
Boc-HN
OH
Ex. 2
1) 6-ethylhydroxyhexanoate, drops 3N HCl, 16-48 hrs.
2) Acetic anhydride, pyridine, MeCN
110
N-NO2
NH2
Boc-HN
O-(CH2)5-CO2Ethyl
Ex. 3
10%-Pd-on-carbon, (EtOH/H2O/HoAc) (4:1:1), H2, psi=40, 16-24 hrs.
120
NH HoAc
NH2
Boc-HN
O-(CH2)5-CO2Ethyl
Ex. 4
1) MeCN, 1N NaOH, pH= 11-13, Allylchloroformate
2) EtOH, 3N LiOH @ r.t.
130
N-Alloc
NH2
Boc-HN
O-(CH2)5-CO2H
Ex. 6
AM resin, BOP or PyBOP, DIEA, DMF
140
N-Alloc
NH2
Boc-HN
O-(CH2)5-CO-NH-AM resin
Ex. 7
1) TFA/Thioanasole/DCM (3:1:6) 15-20 min. @ r.t solutioin & beads dark blue, Ninhydrin Test
2) Coupled P2, P3, P4 amino acids, BOP or PyBOP, DIEA, DMF
150
N-Alloc
NH2
P4-P3-P2-HN
O-(CH2)5-CO-NH-AM resin
Ex. 8
1) (Ph3P)4Pd, (THF/DMSO/.5N HCl/Morpholine) (2:2:1:5)
2) TFA/H2O/DCM (5:1:4), 4 hrs. @ r.t.
160
NH
NH2
P4-P3-P2-HN
OH
Ex. 9; Ex. 10-12

FIGURE 1

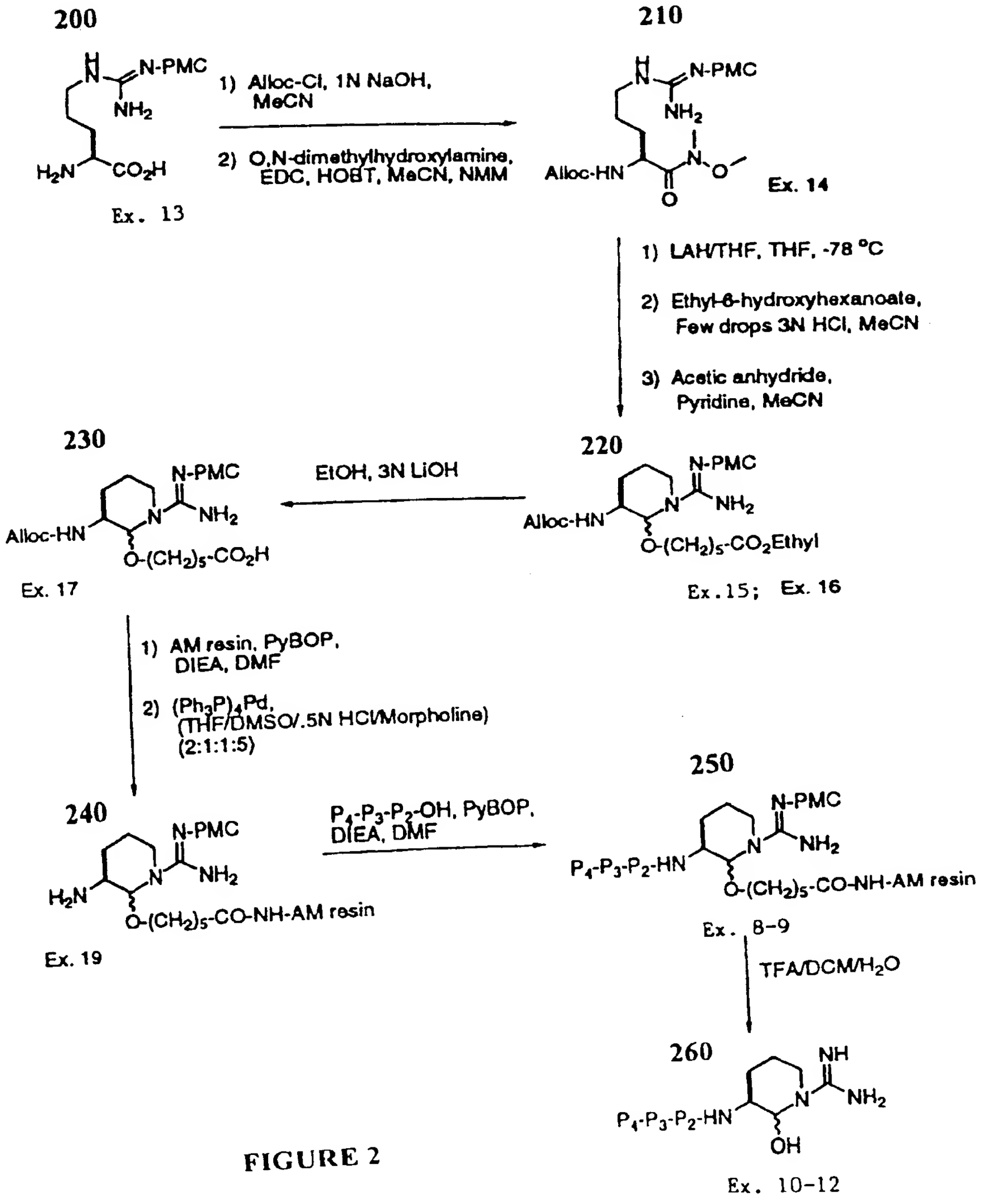
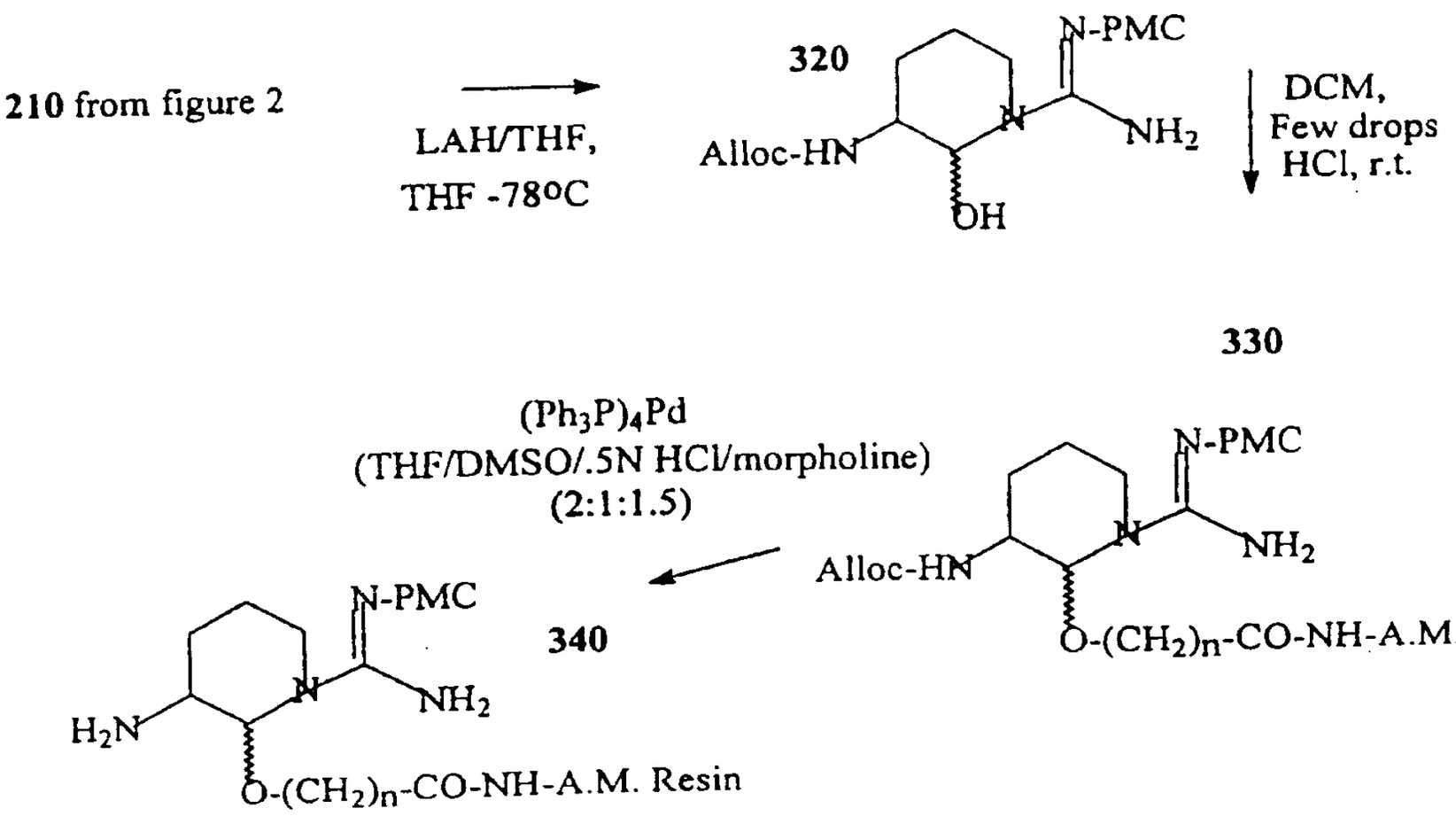

200
N-PMC
NH2
H2N
CO2H
Ex. 13
1) Alloc-Cl, 1N NaOH, MeCN
2) O,N-dimethylhydroxylamine, EDC, HOBT, MeCN, NMM
210
N-PMC
NH2
Alloc-HN
N
O
Ex. 14
1) LAH/THF, THF, -78 °C
2) Ethyl-6-hydroxyhexanoate, Few drops 3N HCl, MeCN
3) Acetic anhydride, Pyridine, MeCN
230
N-PMC
NH2
Alloc-HN
O-(CH2)5-CO2H
Ex. 17
EtOH, 3N LiOH
220
N-PMC
NH2
Alloc-HN
O-(CH2)5-CO2Ethyl
Ex.15; Ex. 16
1) AM resin, PyBOP, DIEA, DMF
2) (Ph3P)4Pd, (THF/DMSO/.5N HCl/Morpholine) (2:1:1:5)
240
N-PMC
NH2
H2N
O-(CH2)5-CO-NH-AM resin
Ex. 19
P4-P3-P2-OH, PyBOP, DIEA, DMF
250
N-PMC
NH2
P4-P3-P2-HN
O-(CH2)5-CO-NH-AM resin
Ex. 8-9
TFA/DCM/H2O
260
NH
NH2
P4-P3-P2-HN
OH
Ex. 10-12

FIGURE 2



(when n=5, this is product **240**)

FIGURE 3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/16901

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D211/56 C07D401/12 C07D405/12 C07K17/08 C07K1/04
A61K31/445

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 731 413 A (RIPKA WILLIAM CHARLES ET AL) 24 March 1998 (1998-03-24) cited in the application claims; example 36	1,25
A	US 5 514 777 A (WEBB THOMAS R ET AL) 7 May 1996 (1996-05-07) cited in the application claims	1,25

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 December 1999	11/01/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	De Jong, B

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INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/US 99/16901

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BAJUSZ S ET AL: "HIGHLY ACTIVE AND SELECTIVE ANTICOAGULANTS: D-PHE-PRO-ARG-H, A FREETRIPEPTIDE ALDEHYDE PRONE TO SPONTANEOUS INACTIVATION, AND ITS STABLE N-METHYL DERIVATIVE, D-MEPHE-PRO-ARG-H" JOURNAL OF MEDICINAL CHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. WASHINGTON, vol. 33, no. 6, page 1729-1735 XP000569685 ISSN: 0022-2623 cited in the application the whole document	1,25
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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